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Draft Aquatic Life Water Quality Criteria for Selenium - 2004

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Aquatic Life Water Quality Criteria for

Selenium

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NOTICES

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Dennis O. McIntyre
Tyler K. Linton
William H. Clement
Gregory J. Smith
Manoel Pacheco
Great Lakes Environmental Center
Columbus, Ohio

Larry T. Brooke
University of Wisconsin-Superior
Superior, Wisconsin

Charles Delos
(document coordinator)
USEPA
Health and Ecological Effects Criteria Division
Washington, D.C.

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Introduction

This document provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) to protect aquatic life from toxic effects of selenium. Under the CWA, States and Tribes are to establish water quality standards to protect designated uses. While this document constitutes the U.S. Environmental Protection Agency's (U.S. EPA) scientific recommendations regarding ambient concentrations of selenium, this document does not substitute for the CWA or U.S. EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally binding requirements on the U.S. EPA, States, Tribes or the regulated community, and might not apply to a particular situation based upon the circumstances. Interested parties are free to raise questions and objections about the substance of this guidance and the appropriateness of the application of this guidance to a particular situation. State and Tribal decision-makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. The U.S. EPA may change this guidance in the future.

This document establishes water quality criteria for protection of aquatic life for selenium. Under Section 304(a) of the CWA, U.S. EPA is to periodically revise water quality criteria to accurately reflect the latest scientific knowledge. Toward this end, a U.S. EPA-sponsored Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation on May 27-28, 1998 brought together experts in selenium research to discuss issues related to the chronic criterion for selenium. As a result of findings from the workshop and the fact that a substantial body of literature on the chronic toxicity of selenium has accumulated since the 1987 document was published, U.S. EPA has decided to update the acute and chronic criteria for selenium.

The criteria presented herein supersede all previous national aquatic life water quality criteria for selenium (U.S. EPA 1976, 1980a, 1987a, 1995).

Selenium Chemistry

Water quality criteria are being derived for total selenium measured as selenite-Se plus selenate-Se, but a variety of forms of selenium can occur in water and tissue. Selenium in aquatic ecosystems exists in a broad range of oxidation states: (+ VI) in selenates ($HSeO_4^-$, SeO_4^{2-}) and selenic acid (H_2SeO_4), (+ IV) in selenites ($HSeO_3^-$, SeO_3^{2-}) and selenous acid (H_2SeO_3), 0 in elemental selenium, and (-II) in selenides (Se^{2-}, HSe^-), hydrogen selenide (H_2Se), and organic selenides (R_2Se). Selenium also shows some

tendency to form catenated species like organic diselenides (RseSeR). Within the normal physiological pH range and the reduction potential range permitted by water, only Se, SeO_3^{2-}, $HSeO_3^-$, and SeO_4^{2-} can exist at thermodynamic equilibrium (Milne 1998). While ionic reactions are expected to be rapid in water, oxidation-reduction reactions may be slow, and the possibility exists for the formation of HSe^- in living systems and some environments where anoxic conditions arise. The parallel behavior of comparable species of sulfur and selenium in living systems has often been observed, but it is important to recognize that their chemical characteristics are different in many ways. For instance, selenate is comparable to chromate in oxidizing strength and far stronger than sulfate [$E^0(SeO_4^{2-}/H_2SeO_3)$ = 1.15 V; $E^0(Cr_2O_7^{2-}/Cr^{3+})$ = 1.33V; $E^0(SO_4^{2-}/H_2SO_3)$ = 0.200V (standard potentials in acid solution: Weast 1969)], whereas selenide is a much stronger reducing agent than sulfide [$E^0(Se/H_2Se)$ = -0.36 V; $E^0[S/H_2S$]= 0.14V)].

Inorganic Selenium

Selenate usually predominates in well-aerated surface waters, especially those with alkaline conditions. In spite of its oxidizing strength, selenate (SeO_4^{2-}) exhibits considerable kinetic stability in the presence of reducing agents (Cotton and Wilkinson 1988). The radius of SeO_4^{2-} is comparable to that of SO_4^{2-} (Frausto da Silva and Williams 1991), and uptake by cells is expected to take place via the same ion channels or permeases for both anions. Competition between sulfate and selenate uptake has been observed in many species: algae (Riedel and Sanders 1996), aquatic plants (Bailey et al., 1995), crustacea (Olge and Knight 1996), fungi (Gharieb et al. 1995), HeLa cells (Yan and Frenkel 1994), and wheat (Richter and Bergmann 1993). Reduced selenate bioconcentration with increasing sulfate concentration has been demonstrated in *Daphnia magna* (Hansen et al. 1993). A significant relationship was shown to exist between acute selenate toxicity to aquatic organisms and ambient sulfate concentrations (Brix et al. 2001a). Competition with selenate has also been observed for phosphate in green algae (Riedel and Sanders 1996), and with chromate and tungstate in anaerobic bacteria (Oremland et al. 1989).

Selenous acid species ($HSeO_3^-$ and SeO_3^{2-}) can predominate in solution under the moderately oxidizing conditions encountered in oxygenated waters. Between pH 3.5 and 9.0 biselenite ion is the predominant ion in water, and at pH values below 7.0, selenites are rapidly reduced to elemental selenium under mildly reducing conditions (Faust 1981), situations that are common in bottom sediments.

Most selenite salts are less soluble than the corresponding selenates. The extremely low solubility of ferric selenite $Fe_2(SeO_3)_3$ ($K_s = 2.0 \pm 1.7 \times 10^{-31}$), and of the basic ferric selenite $Fe_2(OH)_4SeO_3$ ($K_s = 10^{-61.7}$), is important to the environmental cycling of selenium. Selenites also form stable adsorption complexes with ferric oxides, forming complexes of even lower solubility than the ferric selenites. Under certain conditions, selenite (in contrast to selenate) seems to be completely adsorbed in high amounts by ferric hydroxide and, to a lesser extent, by aluminum hydroxide (Faust 1981). Coprecipitation techniques have been applied for preconcentration of selenium in natural waters, using iron (III) hydroxides, which coprecipitates selectively the selenite, but not the selenate, species in river and sea waters (Yoshii et al. 1977). Alum and iron coagulation precipitation can be used in water treatment processes to remove selenite (Clifford et al. 1986). The low levels of selenium in ocean waters have been attributed to the adsorption of selenite by the oxides of metals, such as iron and manganese (National Academy of Sciences 1976).

Relative to selenate, selenite is more reactive because of its polar character, resulting from the asymmetric electron density of the ion, its basicity (attraction to bond with proton), and its nucleophilicity (attraction to bond to a nucleus using the lone pair electrons of the ion). No evidence has yet been presented to show that $HSeO_3^-$ or SeO_3^{2-} is taken up intact into the cell interior. Evidence indicates that selenite is reduced rapidly, even before uptake in some cases, making it difficult to distinguish between uptake and metabolic processes (Milne 1998). Freshwater phytoplankton process selenate and selenite by different mechanisms, leading to different concentrations within the cell, and the concentrations attained are affected by various chemical and biological factors in the environment (Riedel et al. 1991). These authors suggested that selenate is transported into the cell by a biological process with low affinity, whereas selenite appears to be largely physically adsorbed. Contradictory evidence suggesting that selenite uptake is enzymatically mediated was found with marine phytoplankton (Baines and Fisher 2001). Experimental results supporting the hypothesis that separate accumulation mechanisms for selenate and selenite are present in *D. magna* have been published (Maier et al. 1993). However, while some organisms appear to absorb selenite nonspecifically, specific transport systems exist in other species. Sulfate competition is insignificant in *Ruppia maritima* (Bailey et al. 1995), and specific uptake systems have been demonstrated in some microorganisms (Heider and Boeck 1993). Selenite uptake in green algae, unlike selenate, is increased substantially at lower pH values, a property that represents another difference between these two anions (Riedel and Sanders 1996). The uptake of

inorganic selenium species, selenate and selenite, by the green alga *Chlamydomonas reinhardtii* (Dang) was examined as a function of pH over the range 5 to 9, and in media with varying concentrations of major ions and nutrients using ^{75}Se as a radiotracer. Little difference was noted in the uptake of selenate as a function of pH, with the maximum uptake found at pH 8; however, selenite uptake increased substantially at the lower pH values. Differences in speciation are suggested to be the cause of these differences. Selenate exists as the divalent ion SeO_4^{2-} over the range of pH tested; whereas monovalent biselenite ion $HSeO_3^-$ is prevelant at these pH values. At the low end of the pH range, neutral selenous acid may also play a role.

Elemental selenium is not measurably soluble in water. It has been reported that elemental selenium is slowly metabolized by several bacteria (Bacon and Ingledew 1989), and the translocation of elemental selenium into the soft tissue of *Macoma balthica* has been reported (Luoma et al. 1992). The bioavailability of elemental selenium to *M. balthica* was assessed by feeding the organisms ^{75}Se-labeled sediments in which the elemental selenium was precipitated by microbial dissimilatory reduction. A 22% absorption efficiency of particulate elemental selenium was observed. In view of the insolubility of elemental selenium, uptake may be preceeded by air oxidation, or in reducing environments thiols may facilitate the solubilization (Amaratunga and Milne 1994). Elemental selenium can be the dominant fraction in sediments (Zawislanski and McGrath 1998).

Selenium is reduced to hydrogen selenide, H_2Se, or other selenides at relatively low redox potentials. Hydrogen selenide by itself is not expected to exist in the aquatic environment since the Se^0/H_2Se couple falls even below the H^+/H_2 couple. Aqueous solutions of H_2Se are actually unstable in air due to its decomposition into elemental selenium and water. Under moderately reducing conditions, heavy metals are precipitated as the selenides, which have extremely low solubilities. The following are log K_s values of some heavy metal selenides of environmental interest: -11.5 (Mn^{2+}), -26.0 (Fe^{2+}), -60.8 (Cu^+), -48.1 (Cu^{2+}), -29.4 (Zn^{2+}), -35.2 (Cd^{2+}), and -64.5 (Hg^{2+}). The precipitation of selenium as heavy metal selenides can be an important factor affecting the cycling of the element in soils and natural waters.

Organoselenium

Organic selenides (conventionally treated as Se(-II) species) in variable concentrations, usually in the form of free and combined selenomethionine and selenocysteine, are also present in natural surface

waters (Fisher and Reinfelder 1991). Dissolved organic selenides may be an important source of selenium for phytoplankton cells, because they can account for ~80% of the dissolved selenium in open ocean surface waters, and for a significant fraction in many other environments as well (Cutter 1989; Cutter and Cutter 1995). Dissolved organoselenium levels of 14.2%, 65% and 66% were measured in samples (one meter depth) from Hyco Reservoir, NC; Robinson Impoundment, SC; and Catfish Lake, NC; respectively (Cutter 1986). The Hyco Reservoir organoselenium was identified as being protein bound. Organoselenium concentrations were found to range from 10.4% (58.7 µg/L) to 53.7% (1.02 µg/L) of the total selenium present in Lake Creek and Benton Lake, MT surface waters (Zhang and Moore 1996). Organoselenium quite often is measured as the difference between total dissolved selenium and the sum of selenite plus selenate, and is therefore not typically characterized. Much more work is needed in the area of specific identification and characterization of the nature of the organic selenides present in aquatic ecosystems. Organoselenium form(s) are much more bioavailable and probably play a very important role in selenium ecotoxic effects (e.g. Besser et al., 1993; Rosetta and Knight 1995).

Departure from Thermodynamic Equilibrium

In the highly dynamic natural waters, there is often a departure from thermodynamic equilibrium. In the thermodymanic models, kinetic barriers to equilibrium and biological processes are not adequately considered, and the speciation of selenium in oxidized natural waters is not accurately predicted. Selenate is usually the predominate form in solution; however, selenite and organoselenium can both exist at concentrations higher than predicted (Faust 1981; Luoma et al. 1992). Bioaccumulation by microorganisms, bioproduction and release of organoselenium, and mineralization of particulate selenium forms contribute to the disequilibrium.

Physical Distribution of Species in Surface Water

The physical distribution of various selenium species in surface waters is regulated by:

- sorption to or incorporation in suspended particulate matter (SPM), and
- complexation with inorganic and/or organic colloidal material, such as $(FeO \bullet OH)_n$ and humic substances (dissolved organic matter, DOM).

Both sorption to SPM and complexation with colloidal matter reduces the bioavailability of the selenium species. The average fraction of selenium associated with the particulate phase (0.45µm filtration) as determined from eleven different studies of various surface waters was found to be 16% (0-39% range) of the total selenium, i.e., an average operationally defined dissolved selenium level of 84% (Text Table

A). In the James River, VA, the dissolved inorganic and organic selenium was found to be 77% and 70% associated with colloidal matter, respectively (Takayangi and Wong 1984). A study of lake ecosystems in Finland (Wang et al. 1995) found that 52% of the dissolved selenium was associated with humic substances, and in a similar speciation study of Finnish stream waters, Lahermo et al. (1998) determined that 36% of the selenium was complexed with humic matter. Hence, in various waterbodies physical distribution as well as chemical speciation of selenium must be considered in relationship to bioavailability and aquatic toxicity.

Up until recently, the organic selenium fraction has been routinely measured as the difference between total dissolved selenium and the sum of selenite and selenate. Unfortunately, the calculation of this important selenium fraction in water as the difference between the total and measurable inorganic fractions has not permitted this fraction to be fully characterized. New techniques are currently being developed which should help the specific identification and characterization of the nature of the organic selenides present in aquatic systems. This work is particularly important because portions of the organic selenium fraction (e.g., selenomethionine) of total dissolved selenium in water have been shown to be much more bioavailable than the other forms of selenium, and therefore this work is also important for understanding the manifestation of selenium ecotoxic effects.

Sources of Selenium to Aquatic Systems

Selenium occurs in many soil types and enters ground and surface waters through natural weathering process such as erosion, leaching and runoff. The national average concentration of selenium in non-seleniferous surface water ranges from 0.1 to 0.4 µg Se/L (Maier and Knight 1993). Elevated levels of selenium occur in surface waters when substantial quantities of selenium enter surface waters from both natural and anthropogenic sources. It is abundant in the alkaline soils of North America from the Great Plains. Some ground waters in California, Colorado, Kansas, Oklahoma, South Dakota and Wyoming contain elevated concentrations of selenium due to weathering of and leaching from rocks and soils. Ecological impacts have been observed where selenium is concentrated through irrigation practices in areas with seleniferous soils. Selenium also occurs in sulfide deposits of copper, lead, mercury, silver and zinc and can be released during the mining and smelting of these ores. In addition, selenium occurs naturally in coal and fuel oil and is emitted in flue gas and in fly ash during combustion. Some selenium then enters surface waters in drainage from fly-ash ponds and in runoff from fly-ash deposits on land. Notable examples of systems that have been affected by selenium originating from coal ash include

Belews Lake, NC, where 16 of the 20 species originally present were eliminated within a few years after discharge began, and Hyco Reservoir, NC, where selenium toxicity was associated with fish larval mortality (Gillespie and Baumann 1986).

Text Table A. Particulate and dissolved selenium as a function of total selenium in freshwater and marine aquatic ecoystems.

Reference	Waterbody	Particulate Se (% of Total)	Fraction dissolved, fd
Cutter 1989	Carquinezitist, CA	20 - 40	0.6 - 0.8
Cutter 1986	Hyco Reservoir, NC	0	1
Tanizaki et al. 1992	Japanese Rivers	16	0.84
Luoma et al. 1992	San Francisco Bay, CA	22 - 31	0.69-0.78
Cumbie and VanHorn, 1978	Belews Lake, NC	8	0.92
GLEC 1997	Unnamed Stream, Albright, WV	4	0.96
Wang et al. 1995	Finnish Lakes	10	0.9
Lahermo et al. 1998	Finnish Streams	8	0.92
Hamilton et al. 2001ab	Adobe Creek, Fruita, CO	18	0.82
Hamilton et al. 2001a,b	North Pond, Fruita, CO	0	1
Hamilton et al. 2001a,b	Fish Ponds, Fruita, CO	7	0.93
Nakamoto and Hassler 1992	Merced River, CA	0	1
Nakamoto and Hassler 1992	Salt Slough, CA	4	0.96
Welsh and Maughan 1994	Cibola Lake, CA	39	0.62
Welsh and Maughan 1994	Hart Mine Marsh, Blythe, CA	6	0.94
Welsh and Maughan 1994	Colorado River, Blythe, CA	11	0.89
Welsh and Maughan 1994	Palo Verda Oxbow Lake, CA	33	0.67
Welsh and Maughan 1994	Palo Verda Oufall Drain, CA	0	1
Welsh and Maughan 1994	Pretty Water Lake, CA	21	0.79

Selenium Biogeochemistry

The current understanding of the biogeochemistry of selenium has recently been reviewed by Fan et al. (2002). Their review clearly shows the extreme complexity of selenium biogeochemistry in aquatic environments. Fan et al. describe the selenium biogeochemical cycle as follows: dissolved selenium oxyanions are primarily absorbed by aquatic producers, including microphytes and bacteria, and biotransformed into organoselenium form(s) and selenium element (Se^{O}). These, together with other particle-bound selenium sources, constitute the particulate selenium fraction of the water column, and they are poorly understood (Zawislanski and McGrath, 1998). Once accumulated in the aquatic primary and secondary producers, selenium can be transferred through various aquatic consumers (e.g.

zooplankton, insect larvae, larval fish, bivalves) into the top predators, including aquatic birds and piscivorous fish. Selenium can be further chemically transformed through the food chain transfer process.

The microscopic planktonic organisms, including microphytes (cyanobacteria and phytoplankton), bacteria, protozoa, and zooplankton are major components of the particulate matter in the water column. The particulate matter, in turn, forms the basis for detrital materials which can settle onto the sediment, and become the food source for sediment organisms. Suspended particulate matter can also be mineralized in the water column. In addition to this selenium input into the sediment, waterborne selenite and selenate can be physically adsorbed onto the sediment particles, ingested, absorbed, and transformed by the sediment organisms. Sediment-bound selenate and selenite can be reduced to insoluble Se^0 by anaerobic microbial activities. This and water column-derived Se^0 can be reduced further to inorganic and organic selenides (-II form), and/or reoxidized to selenite and selenate by microorganisms in the sediment and/or in the digestive tracts of sediment macroinvertebrates. Selenides can enter the food chain via absorption and/or ingestion (by chironomids or tubificid worms, for example) into sediment organisms, or be oxidized to selenite and selenate. Selenium of different oxidation states can be further biotransformed by sediment organisms and transferred up the food chain. Selenium biotransformation, bioaccumulation, and transfer through both sediment and water column foodwebs constitute the major biogeochemical pathways in aquatic ecosystems.

In addition to accumulating selenium into the biomass, the aquatic producers are the main factors controlling the volatilization of selenium via the production of methylated selenides including, dimethylselenide (DMSe) and dimethyldiselenide (DMDSe). These methylated selenides can be oxidized to selenite, or can exit the water column into the atmosphere. Selenium volatilization into the atmosphere may represent an important process responsible for significant loss of selenium in some aquatic systems. Methylated selenides can also be generated from dissolved selenonium precursor(s) released by aquatic producers into the water. Moreover, other organoselenium forms can be released into the water by aquatic producers, and are reoxidized to selenite and/or reabsorbed by aquatic producers.

Narrow Margin Between Sufficiency and Toxicity

Of all the priority and non-priority pollutants, selenium has the narrowest range of what is beneficial for biota and what is detrimental. Selenium is an essential element required as a mineral cofactor in the biosynthesis of glutathione peroxidases. All of the classic glutathione peroxidases contain selenium and

are found to be involved in the catalytic reaction of these many enzymes (Allan 1999). The major function of the glutathione peroxidases was found to involve the reduction of hydrogen peroxide to water at the expense of the oxidation of glutathione, the enzyme's cofactor. Aquatic and terrestrial organisms require 0.5 µg/g dry weight (dw) of selenium in their diet to sustain metabolic processes, whereas concentrations of selenium that are only an order of magnitude greater than the required level have been shown to be toxic to fish. Selenium deficiency has been found to affect humans (U.S. EPA 1987a), sheep and cattle (U.S. EPA 1987a), deer (Oliver et al. 1990) fish (Thorarinsson et al. 1994; Wang and Lovell 1997; Wilson et al. 1997; U.S. EPA 1987a), aquatic invertebrates (Audas et al. 1995; Caffrey 1989; Cooney et al. 1992; Cowgill 1987; Cowgill and Milazzo 1989; Elendt 1990; Elendt and Bais 1990; Harrison et al. 1988; Hyne et al. 1993; Keating and Caffrey 1989; Larsen and Bjerregaard 1995; Lim and Akiyama 1995; Lindstrom 1991; U.S. EPA 1987a; Winner 1989; Winner and Whitford 1987), and algae (Doucette et al. 1987; Keller et al. 1987; Price 1987; Price et al. 1987; Thompson and Hosja 1996; U.S. EPA 1987a; Wehr and Brown 1985).

Selenium has been shown to mitigate the toxic effects of arsenic, cadmium, copper, inorganic and organic mercury, silver, ofloxacin, methyl parathion and the herbicide paraquat to biota in both aquatic and terrestrial environments (Bjerregaard 1988a, b; Cuvin and Furness 1988; Ding et al. 1988; Krizkova et al. 1996; Malarvizhi and Usharani 1994; Micallef and Tyler 1987; Patel et al. 1988; Paulsson and Lundbergh 1991; Pelletier 1986b, 1988; Phillips et al. 1987; Ramakrishna et al. 1988; Rouleau et al. 1992; Salte et al. 1988; Siegel et al. 1991; Szilagyi et al. 1993; U.S. EPA 1987a). Selenium pretreatment resulted in reduced effects in 128-hr old, but not 6-hr old, embryos of *Oryzias latipes* from cadmium and mercury, whereas prior exposure to selenium did not affect the sensitivity of white suckers to cadmium (U.S. EPA 1987a). In contrast, Birge et al. and Huckabee and Griffith reported that selenium and mercury acted synergistically in producing toxic effects to fish embryos (U.S. EPA 1987a). Selenium is reported to reduce the uptake of mercury by some aquatic species (Southworth et al. 1994; U.S. EPA 1987a), to have no effect on uptake of mercury by a mussel, and to increase the uptake of mercury by mammals and some fish (U.S. EPA 1987a). Selenium augmented accumulation of cadmium in some tissues of the shore crab, *Carcinus maenas* (U.S. EPA 1987a). The available data do not show whether the various inorganic and organic compounds and oxidation states of selenium are equally effective sources of selenium as a trace nutrient, or as reducing the toxic effects of various pollutants.

Selenium Document Information

All concentrations reported herein are expressed as selenium, not as the chemical tested. Although Se(VI) is expected to be the predominant oxidation state at chemical equilibrium in oxygenated alkaline waters, the rate of conversion of Se(IV) to Se(VI) seems to be slow in most natural waters. Therefore, it was assumed that when Se(IV) was introduced into stock or test solutions, it would persist as the predominate state throughout the test, even if no analyses specific for the Se(IV) oxidation state were performed. Similarly, it was assumed that when Se(VI) was introduced into stock or test solutions, it would persist as the predominant state throughout the test, even if no analyses specific for Se(VI) were performed.

An understanding of the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985), hereafter referred to as the Guidelines, and the response to public comments (U.S. EPA 1985a) is helpful for understanding the derivation of the acute criteria for selenium. Briefly, the Guidelines procedure involves the following steps: (1) Acute toxicity test data is gathered from all suitably conducted studies. Data are to be available for species in a minimum of eight families representing a diverse assemblage of taxa. (2) The Final Acute Value (FAV) is derived by extrapolation or interpolation to a hypothetical genus more sensitive than 95 percent of a diverse assemblage of taxa. The FAV, which represents an LC_{50} or EC_{50}, is divided by two in order to obtain an acute criterion protective of nearly all individuals in such a genus. (3) Chronic toxicity test data (longer-term survival, growth, or reproduction) are needed for at least three taxa. Most often the chronic criterion is set by determining an appropriate acute-chronic ratio (the ratio of acutely toxic concentrations to the chronically toxic concentrations) and applying that ratio to the FAV from the previous step. (4) When necessary, the acute and/or chronic criterion may be lowered to protect critically important species.

The chronic criteria procedure explicitly set forth in the Guidelines (Step 3 above) is not well suited to bioaccumulative contaminants for which diet is the primary route of aquatic life exposure. Consequently, that procedure was not used for deriving the chronic criterion for selenium either in the original 1987 criteria document or in this update. Rather, to accord with other provisions of the Guidelines, it was necessary to apply what the Guidelines refer to as “appropriate modifications” of the procedures in order to obtain a criterion “consistent with sound scientific evidence”, as will be described in a later section.

Results of such intermediate calculations as recalculated LC_{50} values and Species Mean Acute Values are given to four significant figures to prevent roundoff error in subsequent calculations, not to reflect the precision of the value. The latest comprehensive literature search for information for this document was conducted in August 2001; some more recent information was included.

The body of this document contains only the information on acute and chronic toxicity of selenium that is relevant to the derivation of the acute and chronic criteria. Supporting information on the toxicity and bioaccumulation of selenium, and the data that were reviewed and not used in deriving the criteria are provided in appendices and include: sulfate correction of selenate acute toxicity (Appendix A); toxicity to aquatic plants (Appendix B); bioconcentration and bioaccumulation (Appendix C); environmental factors affecting selenium toxicity and bioaccumulation (Appendix D); site-specific considerations (Appendix E); other data (Appendix F); unused data (Appendix G); tissue relationships (Appendix H); chronic data summaries (Appendix I); and background Se levels (Appendix J).

Acute Toxicity of Selenite

Data that may be used, according to the Guidelines, in the derivation of Final Acute Values for selenite are presented in Tables 1a and 1b. The following text presents a brief overview of the acceptable data obtained for selenite, followed by a discussion of the more sensitive, and commercially and recreationally important species. A ranking of the relative sensitivity of selenite to selenate for each genera is listed in Tables 2a and 2b.

Acute Toxicity of Se(IV) to Freshwater Animals

Acceptable data on the acute effects of selenite in freshwater are available for 14 species of invertebrates and 20 species of fish (Table 1a). These 34 species satisfy the eight family provision specified in the Guidelines. Invertebrates are both the most sensitive and the most tolerant freshwater species to selenite with Species Mean Acute Values (SMAV) ranging from 440 µg/L for the crustacean, *Ceriodaphnia dubia*, to 203,000 µg/L for the leech, *Nephelopsis obscura*. The selenite SMAVs for fishes range from 1,783 µg/L for the striped bass, *Morone saxatilis*, to 35,000 µg/L for the common carp, *Cyprinus carpio*. The following text presents a species-by-species discussion of the eight most sensitive genera, plus all commercially and recreationally important species.

Hyalella (amphipod)

The most sensitive freshwater genus is the amphipod, *Hyalella*, with a Genus Mean Acute Value (GMAV) of 461.4 µg Se/L. The GMAV is derived from five 96-hr acute flow-through measured tests where the LC_{50} values ranged from 340 to 670 µg Se/L (GLEC 1998; Halter et al. 1980). A sixth test conducted under non flow-through conditions is also listed in Table 1a (Brasher and Ogle 1993), but the Guidelines recommend using flow-through measured data in preference to static or renewal data.

Ceriodaphnia (cladoceran)

The second most sensitive freshwater genus is *Ceriodaphnia*, with a GMAV of <515.3 µg Se/L that is derived from the geometric mean of the *C. affinis* (<603.6 µg Se/L) and *C. dubia* (440 µg Se/L) SMAVs. Four static unmeasured 48-hr studies are available for *C. affinis* where the LC_{50} values ranged from <480 to 720 µg Se/L (Owsley 1984; Owsley and McCauley 1986). The one available *C. dubia* acute study was conducted by GLEC (1999) that exposed <24-hr old neonates to sodium selenite for 48 hours under flow-through measured conditions. The resultant 48-hr LC_{50} value was 440 µg Se/L, which is the most sensitive SMAV for selenite in the database.

Daphnia (cladoceran)

The eleven available acute values are used to calculate the *Daphnia magna* SMAV of 905.3 µg Se/L (acute LC_{50} values ranged from 215 to 3,020 µg Se/L), but only one flow-through measured acute LC_{50} test value of 1,987 µg Se/L is used for the for *D. pulex* SMAV (a second static measured test conducted by Reading (1979) is listed, but not used to calculate the SMAV) . The resultant GMAV of 1,341 µg Se/L for *Daphnia* is the third most sensitive for selenite.

Hydra

The fourth most sensitive freshwater genus is *Hydra*, with a GMAV of 1,700 µg Se/L. The GMAV is derived from the one available static-measured test conducted by Brooke et al. (1985).

Morone (striped bass)

Two 96-hr static unmeasured tests are available for the striped bass, *Morone saxatilis,* and the LC_{50} values were 1,325 and 2,400 µg Se/L (Palawski et al. 1985). The geometric mean of the two values yield the GMAV of 1,783 µg Se/L.

Pimephales (fathead minnow)

A total of 16 fathead minnow acute studies are presented in Table 1a, but only the eight flow-through measured LC_{50} values are used to derive the GMAV of 2,209 µg Se/L. The eight flow-through LC_{50} values ranged from 620 to 5,200 µg Se/L (Cardwell et al. 1976a,b; GLEC 1998; Kimball manuscript).

Gammarus (amphipod)

The seventh most sensitive freshwater genus is *Gammarus*, with a GMAV of 3,489 µg Se/L that is derived from the geometric mean of five flow-through measured studies (GLEC 1998, 1999) where the LC_{50} values ranged from 1,800 to 10,950 µg Se/L. Two static measured acute studies were conduced by Brooke et al. (1985) and Brooke (1987), but as recommended by the Guidelines, were not used to calculate the SMAV for this species.

Jordanella (flagfish)

The eighth most sensitive freshwater genus is *Jordanella*, with a GMAV of 6,500 µg Se/L. The GMAV is derived from the one available 96-hr flow-through measured test conducted by Cardwell et al. (1976a,b) that exposed *Jordanella floridae* to selenium dioxide.

Oncorhynchus (salmonid)

The GMAV of 10,580 µg Se/L for the commercially important salmonid *Oncorhynchus* is derived from the geometric mean of the coho salmon (O. *kisutch*; 7,240 µg Se/L), chinook salmon (*O. tshawytscha*; 15,596 µg Se/L) and rainbow trout (*O. mykiss*; 10,488 µg Se/L) SMAVs. Three static unmeasured 96-hr studies are used to calculate the coho salmon SMAV where the LC_{50} values ranged from 3,578 to 13,600 µg Se/L (Hamilton and Buhl 1990b; Buhl and Hamilton 1991). A fourth coho salmon LC_{50} value is available for an acute test initiated with the tolerant alevin life stage (Buhl and Hamilton 1991), but based on Guideline recommendations this value is not used when data are available from a more sensitive life stage.

Six acute chinook salmon static unmeasured 96-hr acute studies conducted with the more sensitive post-alevin life stage of the fish are used to determine the 15,596 µg Se/L SMAV for the species and the LC_{50} values ranged from 8,150 to 23,400 µg Se/L (Hamilton and Buhl 1990b). The two acute studies conducted with the tolerant eyed egg and alevin life stages by the same authors are not used in the SMAV determination as recommended by the Guidelines. Hamilton and Buhl (1990b) noted that chinook salmon fry were consistently more sensitive than either the embryos or alevin to selenite.

A total of seven rainbow trout acute studies are presented in Table 1a, but only the two flow-through measured LC_{50} values are used to derive the SMAV of 10,488 µg Se/L as recommended by the Guidelines. The two 96-hr flow-through test LC_{50} values are 8,800 and 12,500 µg Se/L (Goettl and Davies 1976; Hodson et al. 1980). As with the coho and chinook salmon, the alevin life stage was less sensitive to selenite.

Lepomis (bluegill)

The GMAV of 28,500 µg Se/L for the recreationally important bluegill sunfish, *Lepomis macrochirus*, is derived from the 96-hr flow-through measured test conducted by Cardwell et al. (1976a,b). The static measured acute study conduced by Brooke et al. (1985) was not used to calculate the SMAV for this species, as recommended by the Guidelines.

Se(IV) Freshwater Final Acute Value Determination

Freshwater Species Mean Acute Values (Table 1a) were calculated as geometric means of the available acute values for selenite, and Genus Mean Acute Values (Table 2a) were then calculated as geometric

means of the Species Mean Acute Values. Of the 28 genera for which freshwater mean acute values are available, the most sensitive genus, *Hyalella*, is 440 times more sensitive than the most tolerant, *Nephelopsis*. The range of sensitivities of the four most sensitive genera spans a factor of 3.7. The freshwater Final Acute Value (FAV), representing the most sensitive 5th percentile genus, is calculated to be 514.9 µg/L for selenite using the procedure described in the Guidelines and the Genus Mean Acute Values in Table 2a. The Final Acute Value is higher than the lowest Species Mean Acute Value (Figure 1).

Acute Toxicity of Se(IV) to Saltwater Animals

Acute toxicity data that can be used to derive a saltwater criterion for selenite are available for 10 species of invertebrates and eight species of fish that are resident in North America (Table 1b). These 18 species satisfy the eight family provision specified in the Guidelines. The range of SMAVs for saltwater invertebrates extends from 255 µg Se/L for juveniles of the bay scallop, *Argopecten irradians* (Nelson et al. 1988) to greater than 10,000 µg Se/L for embryos of the blue mussel, *Mytilus edulis* (Martin et al. 1981) and embryos of the Pacific oyster, *Crassostrea gigas* (Glickstein 1978; Martin et al. 1981). The range of SMAVs for fish is slightly wider than that for invertebrates, extending from 599 µg Se/L for larvae of the haddock, *Melanogrammus aeglefinus*, to 17,350 µg Se/L for adults of the fourspine stickleback, *Apeltes quadracus* (Cardin 1986). No consistent relationship was detected between life stage of invertebrates or fish and their sensitivity to selenite, and few data are available concerning the influence of temperature or salinity on the toxicity of selenite to saltwater animals. Acute tests with the copepod, *Acartia tonsa*, at 5 and 10°C gave similar results (Lussier 1986). The following text presents a species-by-species discussion of the eight most sensitive genera, plus all commercially and recreationally important species. The genera sensitivity ranking is listed in Table 2b.

<u>*Argopecten* (bay scallop)</u>

The most sensitive saltwater genus is *Argopecten*, with a GMAV of 255 µg Se/L. The GMAV is derived from the one available bay scallop (*Argopecten irradians)* static-renewal unmeasured test conducted by Nelson et al. (1988) at a salinity of 25 g/kg.

Melanogrammus (haddock)

The second most sensitive saltwater genus is *Melanogrammus*, with a GMAV of 599 µg Se/L. The GMAV is derived from the one available haddock (*Melanogrammus aeglefinus*) static unmeasured test conducted by Cardin (1986) at a salinity of 30 g/kg.

Cancer (dungeness crab)

The third most sensitive saltwater genus is *Cancer*, with a GMAV of 1,040 µg Se/L. The GMAV is derived from the one available static unmeasured test conducted by Glickstein (1978) that exposed *Cancer magister* to selenium oxide at a salinity of 33.8 g/kg.

Penaeus (brown shrimp)

The fourth most sensitive saltwater genus is *Penaeus*, with a GMAV of 1,200 µg Se/L. The GMAV is derived from the one available static unmeasured test conducted by Ward et al. (1981) that exposed *Penaeus aztecus* to sodium selenite at a salinity of 30 g/kg.

Acartia (copepod)

The fifth most sensitive saltwater genus is *Acartia*, with a GMAV of 1,331 µg Se/L that is derived from the geometric mean of the *A. clausi* (2,110 µg Se/L) and *A. tonsa* (839 µg Se/L) SMAVs. Each of the SMAVs is derived from one static unmeasured acute test conducted by Lussier (1986) that exposed each species to selenious acid at a salinity of 30 g/kg.

Americamysis (*Mysidopsis*) (mysid)

The GMAV of 1,500 µg Se/L for the mysid *Americamysis* (formerly *Mysidopsis*) is derived from the one *Americamysis bahia* 96-hr flow-through measured test conducted by Ward et al. (1981). The static unmeasured acute study conduced by U.S. EPA (1978) was not used to calculate the SMAV for this species as recommended by the Guidelines. The flow-through measured test was conducted with selenious acid at a salinity of 15-20 g/kg.

Spisula (surf clam)

The seventh most sensitive saltwater genus is *Spisula*, with a GMAV of 1,900 µg Se/L. The GMAV is derived from the one available static-renewal unmeasured test conducted by Nelson et al. (1988) that exposed *Spisula solidissima* to sodium selenite at a salinity of 25 g/kg.

Morone (striped bass)

Five 96-hr static unmeasured tests are available for the striped bass, *Morone saxatilis,* and the LC_{50} values ranged from 1,550 to 3,900 µg Se/L (Chapman 1992; Palawski et al. 1985). The geometric mean of the five values yielded the GMAV of 3,036 µg Se/L. All the tests were conducted with sodium selenite at a salinity of 1-5 g/kg.

Paralichthys (summer flounder)

The GMAV of 3,497 µg Se/L for the commercially important summer flounder, *Paralichthys dentatus,* is derived from one 96-hr static unmeasured acute test conducted by Cardin (1986) that exposed embryos to selenious acid at a salinity of 30.2 g/kg.

Callinectes (blue crab)

The GMAV of 4,600 µg Se/L for the commercially important blue crab, *Callinectes sapidus,* is derived from one static unmeasured acute test conducted by Ward et al. (1981) that exposed juveniles to sodium selenite at a salinity of 30 g/kg.

Crassostrea (Pacific oyster)

Two static unmeasured tests are available for the commercially important Pacific oyster, *Crassostrea gigas*, and the LC_{50} values were both >10,000 µg Se/L (Glickstein 1978; Martin et al. 1981). The geometric mean of the two values yielded the GMAV of >10,000 µg Se/L. The tests were conducted with selenium oxide and sodium selenite at a salinity of 33.8 g/kg.

Mytilus (blue mussel)

The GMAV for the commercially important blue mussel, *Mytilus edulis*, is also >10,000 µg Se/L, and is derived from the one static unmeasured acute test conducted by Martin et al. (1981) that exposed embryos to selenium oxide at a salinity of 33.8 g/kg.

Pseudopleuronectes (winter flounder)

The GMAV of 14,649 µg Se/L for the commercially important winter flounder, *Pseudopleuronectes americanus,* is derived from two 96-hr static unmeasured acute tests conducted by Cardin (1986) that exposed larvae to selenious acid at a salinity of 28-30 g/kg.

Se(IV) Saltwater Final Acute Value Determination

Of the 17 genera for which saltwater mean acute values are available for selenite (Table 2b), the most sensitive genus, *Argopectin*, is 68 times more sensitive than the most tolerant, *Apeltes*. The sensitivities of the four most sensitive genera differ by a factor of 4.7, and these four include three invertebrates and one fish, of which an invertebrate is the most sensitive of the four. The saltwater Final Acute Value, representing the most sensitive 5th percentile genus, is 253.4 µg/L for selenite, which is slightly lower than the lowest Species Mean Acute Value (Figure 2).

Acute Toxicity of Selenate

Data that may be used, according to the Guidelines, in the derivation of Final Acute Values for selenate are presented in Tables 1a and 1b. The following text presents a brief overview of the acceptable data obtained for selenate, and includes a discussion of the more sensitive and important species. The genera sensitivity ranking is listed in Tables 2a and 2b.

Sulfate-dependent Toxicity of Selenate

The toxicity of a number of metals (e.g., copper and cadmium) to aquatic organisms is related to the concentration of hardness in the water. The toxicity of these metals to many different aquatic species has been shown to decrease as the hardness concentration increases. A similar relationship also has been recognized between selenate and dissolved sulfate in freshwater (a similar relationship is not evident between selenite and sulfate or between either form of selenium and hardness). The studies reviewed in this document indicate that, as the concentration of sulfate increases, the acute toxicity of selenate is reduced (less toxic). Selenate acute toxicity tests conducted at different levels of dissolved sulfate are available with *C. dubia, D. magna, H. azteca, G. pseudolimnaeus,* chinook salmon and fathead minnows (Table 1a). These data indicate that, in general, selenate is more toxic to these species in low sulfate water than in higher sulfate water.

Sulfate Correction

As discussed in the introduction of this document, sulfate has been shown to compete with selenate in their uptake into aquatic organisms (Olge and Knight 1996; Riedel and Sanders 1996; Bailey et al. 1995; Hansen et al. 1993) and affect the acute toxicity of selenate (Brix et al. 2001a). Sulfate is used here as a correction to the toxicity of selenate. However, it should be emphasized that the sulfate adjustment is not a precise measure, but an estimation. The variability associated with different life stages, clones and test conditions of the studies used to determine the sulfate slope all contribute to the uncertainty of the sulfate correction. In selected cases, insensitive life stages were not used in the analysis (e.g., the eyed-egg and alevin test results were not used for the chinook salmon).

Following recommendations in the guidelines (Stephan et al. 1985), an analysis of covariance (Sokal and Rohlf 1981) was implemented in Microsoft Excel to calculate a common slope for regression lines projecting the natural logarithm of selenate LC_{50}s as a function of the natural logarithm of sulfate concentrations. The common regression line is the best estimate of the collective relationship between

toxicity and sulfate concentration. With analysis of covariance, different species will be weighted relative to the number of data points they have. In this case, the fathead minnow has 18 data points out of the total of 57, the next most frequent species, *C. dubia,* has 13 data points, and the four remaining species have eight or fewer data points.

This analysis of covariance model was fit to the selenate data in Table 1a for the six species for which definite acute values ("less than" or "greater than" values were not used) were available over a range of sulfate levels, such that the highest sulfate value was at least three times the lowest, and the highest was also at least 100 mg/L higher than the lowest (other species in Table 1a either did not meet these criteria or did not show any sulfate-toxicity trend due to differences in exposure methods, species, age, etc.). A list of the species and acute toxicity-sulfate values used to estimate the acute sulfate slope is provided in Appendix A.

Regression analysis revealed significant, positive slopes for five of six species that had acute values precisely determined. The slopes for all six species ranged from 0.19 to 0.87, and the common slope for these six species was 0.5812. An F-test was used to test the null hypothesis that slopes of all species were equal. This test revealed that the null hypothesis could not be rejected ($F_{5,45} = 2.82$, $P>0.05$). Individual slopes were not significantly different than the overall pooled slope (Tukey test, all $|q| <3.3$, $q_{0.05,(2),47,7} = 4.39$). Analysis of covariance thus confirmed that it is correct to assume that there is no significant variation in slopes among species, and that the overall slope is a reasonable estimate of the relationship between sulfate concentration and selenate toxicity.

The pooled slope of 0.5812 was used to adjust the freshwater selenate acute values in Table 1a to a sulfate level of 100 mg/L, except where it was not possible because no sulfate value was reported. Species Mean Acute Values (SMAV) were calculated as geometric means of the adjusted acute values (only the underlined EC50/LC50 species values were used to calculate the respective SMAV). As stated in the Guidelines (Stephen et al. 1985), flow-through measured study data are normally given preference over non-flow-through data for a particular species. In certain cases flow-through measured results were available, yet preference was given to the sensitive life stage for certain species in calculating SMAVs. Genus Mean Acute Values (GMAV) at a sulfate level of 100 mg/L were then calculated (Table 1a) as geometric means of the available freshwater Species Mean Acute Values and ranked (Table 2a).

Acute Toxicity of Se(VI) to Freshwater Animals (Sulfate Adjusted Values)

Acceptable data on the acute effects of selenate in freshwater are available for 12 invertebrate species and 11 species of fish (Table 1a). These 23 species satisfy the eight family provision of the Guidelines. Invertebrates are both the most sensitive and the most tolerant freshwater species to selenate with sulfate adjusted SMAVs ranging from 593 µg/L for the crustacean, *Daphnia pulicaria*, to 1,515,616 µg/L for the leech, *Nephelopsis obscura*. The selenate SMAVs for fishes range from 10,305 µg/L for the razorback sucker, *Xyrauchen texanus*, to 226,320 µg/L for channel catfish, *Ictalurus punctatus*. The following text presents a species-by-species discussion of the eight most sensitive genera, plus all commercially and recreationally important species.

Ceriodaphnia (cladoceran)

The most sensitive freshwater genus is the cladeceran, *Ceriodaphnia*, with a sulfate adjusted GMAV of 842 µg Se/L. The GMAV is derived from one 48-hr acute flow-through measured test (GLEC 1999). Twelve additional tests conducted under non flow-through conditions are also listed in Table 1a (Brix et al. 2001a,b), but the Guidelines recommend using flow-through measured data in preference to static or renewal data.

Hyalella (amphipod)

The second most sensitive freshwater genus is the amphipod, *Hyalella*, with a sulfate adjusted GMAV of 1,397 µg Se/L. The GMAV is derived from four 96-hr acute flow-through measured tests where the LC_{50} values ranged from 723 to 4,224 µg Se/L (GLEC 1998). Three tests conducted under non flow-through conditions are also listed in Table 1a (Adams 1976; Brasher and Ogle 1993; Brix et al. 2001a,b), but are not used to calculate the SMAV as recommended by the Guidelines.

Daphnia (cladoceran)

The third most sensitive freshwater genus is *Daphnia*, with a sulfate adjusted GMAV of 1,887 µg Se/L that is derived from the geometric mean of the *D. magna* (3,314 µg Se/L), *D. pulex* (3,420 µg Se/L) and *D. pulicaria* (593 µg Se/L) SMAVs. Five static and one static-renewal measured 48-hr studies are available for *D. magna* where the LC_{50} values ranged from 1,955 to 5,093 µg Se/L (Boyum 1984; Brooke et al. 1985; Dunbar et al. 1983; Ingersol et al. 1990; Maier et al. 1993).

The *D. pulex* SMAV of 3,420 µg Se/L is based on the 48-hr flow-through measured test conducted by GLEC (1999) that exposed <24-hr old neonates to sodium selenate. Two static measured tests conducted by Brix et al. (2001a,b), are not used to calculate the SMAV as recommend by the Guidelines.

The one available *D. pulicaria* acute study was conducted by Boyum (1984) that exposed neonates to sodium selenate for 48 hours under static measured conditions. The resultant 48-hr LC_{50} value was 593 µg Se/L, which is the most sensitive SMAV for selenate in the database.

Gammarus (amphipod)

The fourth most sensitive freshwater genus is *Gammarus*, with a sulfate adjusted GMAV of 2,522 µg Se/L that is derived from the geometric mean of the *G. lacustris* (2,747 µg Se/L) and *G. pseudolimnaeus* (2,315 µg Se/L) SMAVs. The static measured acute test conduced by Brix et al. (2001a,b) is the only LC_{50} value available for *G. lacustris*.

The *G. pseudolimnaeus* SMAV of 2,315 µg Se/L is based on five 96-hr flow-through measured tests conducted by GLEC (1998, 1999). Two static measured acute studies were conduced by Brooke et al. (1985) and Brooke (1987), but as recommended by the Guidelines, were not used to calculate the SMAV for this species.

Xyrauchen (razorback sucker)

Six 96-hr static unmeasured tests are available for the razorback sucker, *Xyrauchen texanus*, and the LC_{50} values ranged from 7,839 to 16,184 µg Se/L (Buhl and Hamilton 1996; Hamilton 1995; Hamilton and Buhl 1997a). The geometric mean of the six values yield the GMAV of 10,309 µg Se/L.

Gila (bonytail)

The sixth most sensitive freshwater genus is *Gila*, with a sulfate adjusted GMAV of 10,560 µg Se/L. The GMAV is derived from the one static-unmeasured test conducted with the more sensitive larval stage (Buhl and Hamilton 1996). Four other static-unmeasured tests were conducted with less sensitive life stages, but as recommended by the Guidelines, the results were not used to calculate the SMAV for this species.

Pimephales (fathead minnow)

A total of nine fathead minnow acute studies are presented in Table 1a, but only the five flow-through measured LC_{50} values are used to derive the sulfate adjusted GMAV of 11,346 µg Se/L. The five flow-through LC_{50} values ranged from 7,286 to 18,860 µg Se/L (Spehar 1986; GLEC 1998). The four static tests are not used to calculate the SMAV as recommended by the Guidelines.

Ptychocheilus (Colorado squawfish)

The eighth most sensitive freshwater genus is *Ptychocheilus* with a sulfate adjusted GMAV of 18,484 µg Se/L. The GMAV is derived from the three static-unmeasured test conducted with the sensitive life stage of *Ptychocheilus lucius* (Buhl and Hamilton 1996; Hamilton 1995). Three other static-unmeasured tests were conducted with less sensitive life stages, but as recommended by the Guidelines, the results were not used to calculate the SMAV for this species.

Oncorhynchus (salmonid)

The sulfate adjusted GMAV of 47,164 µg Se/L for the commercially important salmonid *Oncorhynchus* is derived from the geometric mean of the coho salmon (*O. kisutch*; 29,141 µg Se/L), chinook salmon (*O. tshawytscha*; 83,353 µg Se/L) and rainbow trout (*O. mykiss*; 43,192 µg Se/L) SMAVs. Three static unmeasured 96-hr studies are used to calculate the coho salmon SMAV where the LC_{50} values ranged from 20,963 to 51,935 µg Se/L (Buhl and Hamilton 1991; Hamilton and Buhl 1990b). A fourth coho salmon LC_{50} value is available for an acute test initiated with the tolerant alevin life stage (Buhl and Hamilton 1991), but based on Guideline recommendations this value is not used when data are available from a more sensitive life stage.

Five acute chinook salmon static unmeasured 96-hr acute studies conducted with the more sensitive life stage of the fish are used to determine the sulfate adjusted 83,353 µg Se/L SMAV for the species with LC_{50} values ranging from 69,939 to 97,550 µg Se/L (Hamilton and Buhl 1990b). The two acute studies conducted with the tolerant eyed egg and alevin life stages by the same authors are not used in the SMAV determination as recommended by the Guidelines.

A total of four rainbow trout acute studies are presented in Table 1a, but only the results from the two static tests conducted with the sensitive juvenile life stage were used to calculate the SMAV of 43,192 µg Se/L (Brooke et al. 1985; Buhl and Hamilton 1991). The two test results obtained with less sensitive life stages were not used as recommended by the Guidelines.

Lepomis (bluegill)

The sulfate adjusted GMAV of 216,033 µg Se/L for the recreationally important bluegill sunfish, *Lepomis macrochirus*, is derived from the 96-hr static measured test conducted by Brooke et al. (1985) that exposed juvenile bluegill to sodium selenate.

Ictalurus (channel catfish)

The sulfate adjusted GMAV of 226,320 µg Se/L for the commercially important channel catfish, *Ictalurus punctatus*, is derived from the 96-hr static measured test conducted by Brooke et al. (1985) that exposed juvenile catfish to sodium selenate.

Se(VI) Freshwater Final Acute Value Determination

Of the 18 freshwater genera for which mean sulfate adjusted acute values are available for selenate, the most sensitive, *Ceriodaphnia*, is 1,800 times more sensitive than the most tolerant, *Nephelopsis*. The range of sensitivities of the four most sensitive genera, all invertebrates, spans a factor of 3.0.

At a sulfate level of 100 mg/L, the freshwater Final Acute Value, representing the most sensitive 5th percentile genus, was calculated to be 834.4 µg/L for selenate. This Final Acute Value is lower than the acute value of the most sensitive freshwater species (Table 2a and Figure 3). The resultant freshwater Criterion Maximum Concentration (CMC) for selenate (in µg/L) = $e^{(0.5812[\ln(\text{sulfate})]+3.357)}$. At a sulfate level of 100 mg/L this yields 417.2 µg/L, or one-half the FAV.

Acute Toxicity of Se(VI) to Saltwater Animals

The only species with which acute tests have been conducted on selenate in salt water is the striped bass (Table 1b). Klauda (1985a, b) obtained 96-hr selenate LC_{50} values of 9,790 and 85,840 µg/L using flow-through measured methodology with prolarvae and juvenile striped bass, respectively. In static unmeasured tests, Chapman (1992) determined selenate 96-hr LC_{50} values that ranged from 23,700 to 29,000 µg/L using 24 to 32 day posthatch striped bass larvae. The more sensitive prolarvae life stage test conducted under flow-through conditions is used to yield the SMAV and GMAV of 9,790 µg Se/L for the striped bass.

Se(VI) Saltwater Final Acute Value Determination

The one saltwater species available for selenate does not satisfy the eight family provision specified in the Guidelines. Therefore, a saltwater Final Acute Value for selenate cannot be determined.

Comparison of Selenite and Selenate Acute Toxicity

Species Mean Acute Values have been determined for both selenite and selenate with 20 freshwater species (Table 3a) and one saltwater species (Table 3b). Of these 21 species, 20 are more sensitive to Se(IV). Only the amphipod, *Gammarus pseudolimnaeus*, is more sensitive to Se (VI), and is in the sensitive portion of the Table 3a distribution. Consistent with the acute toxicity sensitivity pattern, the FAV for Se(VI) is higher than the FAV for Se (IV).

Table 1a. Acute Toxicity of Selenium to Freshwater Animals

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
FRESHWATER SPECIES						
Selenite						
Hydra (adult), *Hydra sp.*	S, M	Sodium selenite	-	**1,700**	1,700	Brooke et al. 1985
Worm, *Tubifex tubifex*	R, U	Sodium selenite	245	**7,710**	7,710	Khangarot 1991
Leech (adult), *Nephelopsis obscura*	S, M	Sodium selenite	49.8	**203,000**	203,000	Brooke et al. 1985
Snail (adult), *Aplexa hypnorum*	S, M	Sodium selenite	50.6	**53,000**	-	Brooke et al. 1985
Snail (adult), *Aplexa hypnorum*	S, M	Sodium selenite	49.8	**23,000**	34,914	Brooke et al. 1985
Snail, *Physa sp.*	S, U	Sodium selenite	45.7	**24,100**	24,100	Reading 1979
Cladoceran (<24 hr), *Ceriodaphnia dubia*	F, M	Sodium selenite	127 (sulfate=25)	**440**	440	GLEC 1999
Cladoceran (<24 hr), *Ceriodaphnia affinis*	S, U	Sodium selenite	100.8	**600**	-	Owsley 1984; Owsley and McCauley 1986
Cladoceran (36-60 hr), *Ceriodaphnia affinis*	S, U	Sodium selenite	100.8	**720**	-	Owsley 1984
Cladoceran (84-108 hr), *Ceriodaphnia affinis*	S, U	Sodium selenite	100.8	**640**	-	Owsley 1984
Cladoceran (72-120 hr), *Ceriodaphnia affinis*	S, U	Sodium selenite	100.8	**<480**	<603.6	Owsley 1984
Cladoceran, *Daphnia magna*	S, U	Sodium selenite	214	**2,500**	-	Bringmann and Kuhn 1959a
Cladoceran, *Daphnia magna*	S, U	Selenious acid[c]	72	**430**	-	LeBlanc 1980
Cladoceran, *Daphnia magna*	S, M	Sodium selenite	129.5	**1,100**	-	Dunbar et al. 1983
Cladoceran, *Daphnia magna*	S, M	Sodium selenite	138	**450**	-	Boyum 1984
Cladoceran (<24 hr), *Daphnia magna*	S, U	Sodium selenite	-	**215**	-	Adams and Heidolph 1985
Cladoceran (<24 hr), *Daphnia magna*	S, U	Sodium selenite	40	**870**	-	Mayer and Ellersieck 1986
Cladoceran (<24 hr), *Daphnia magna*	S, U	Sodium selenite	280	**2,370**	-	Mayer and Ellersieck 1986

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Cladoceran, *Daphnia magna*	S, M	Sodium selenite	45.5	**700**	-	Ingersoll et al. 1990
Cladoceran, *Daphnia magna*	S, M	Sodium selenite	136	**3,020**	-	Ingersoll et al. 1990
Cladoceran (<24 hr), *Daphnia magna*	R, M	Sodium selenite	80-100	**550**	-	Maier et al. 1993
Cladoceran, *Daphnia magna*	S, M	Selenious acid	220[d]	**1,220**	9,05.3	Kimball, Manuscript
Cladoceran, *Daphnia pulex*	S, M	Sodium selenite	46.4	3,870	-	Reading 1979; Reading and Buikema 1983
Cladoceran (<24 hr), *Daphnia pulex*	F, M	Sodium selenite	128 (sulfate=25)	**1,987**	1,987	GLEC 1999
Amphipod (adult), *Gammarus pseudolimnaeus*	S, M	Sodium selenite	48.3	4,300	-	Brooke et al. 1985
Amphipod (adult), *Gammarus pseudolimnaeus*	S, M	Sodium selenite	53.6	1,700	-	Brooke 1987
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenite	139 (sulfate=24)	**2,260**	--	GLEC 1998
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenite	137 (sulfate=138)	**3,130**	--	GLEC 1998
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenite	144 (sulfate=326)	**1,800**	--	GLEC 1998
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenite	138 (sulfate=758)	**3,710**	--	GLEC 1998
Amphipod (adult), *Gammarus pseudolimnaeus*	F, M	Sodium selenite	128 (sulfate=25)	**10,950**	3,489	GLEC 1999
Amphipod (2 mm length), *Hyalella azteca*	R, M	Sodium selenite	133	420	-	Brasher and Ogle 1993
Amphipod, *Hyalella azteca*	F, M	Sodium selenite	329	**340**	-	Halter et al. 1980
Amphipod, *Hyalella azteca*	F, M	Sodium selenite	132 (sulfate=64)	**670**	--	GLEC 1998

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Amphipod, *Hyalella azteca*	F, M	Sodium selenite	132 (sulfate=138)	**<350**	--	GLEC 1998
Amphipod, *Hyalella azteca*	F, M	Sodium selenite	138 (sulfate=359)	**<460**	--	GLEC 1998
Amphipod, *Hyalella azteca*	F, M	Sodium selenite	138 (sulfate=642)	**570**	461.4	GLEC 1998
Midge (4th instar), *Chironomus decorus*	R, M	Sodium selenite	85	**48,200**	48,200	Maier and Knight 1993
Midge, *Chironomus plumosus*	S, U	Sodium selenite	39	**24,150**	-	Mayer and Ellersieck 1986
Midge, *Chironomus plumosus*	S, U	Sodium selenite	280	**27,850**	25,934	Mayer and Ellersieck 1986
Midge, *Tanytarsus dissimilis*	F, M	Selenium dioxide	48	**42,500**	42,500	Call et al. 1983
Coho salmon (0.5 g), *Oncorhynchus kisutch*	S, U	Sodium selenite	211	**7,800**	-	Hamilton and Buhl 1990b
Coho salmon (2.6 g), *Oncorhynchus kisutch*	S, U	Sodium selenite	333	**13,600**	-	Hamilton and Buhl 1990b
Coho salmon (alevin), *Oncorhynchus kisutch*	S, U	Sodium selenite	41	35,560[f]	-	Buhl and Hamilton 1991
Coho salmon (juvenile), *Oncorhynchus kisutch*	S, U	Sodium selenite	41	**3,578**	7,240	Buhl and Hamilton 1991
Chinook salmon (0.7 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	211	**14,800**	-	Hamilton and Buhl 1990b
Chinook salmon (0.5 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	211	**13,000**	-	Hamilton and Buhl 1990b
Chinook salmon (1.6 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	333	**23,100**	-	Hamilton and Buhl 1990b
Chinook salmon (1.6 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	333	**23,400**	-	Hamilton and Buhl 1990b
Chinook salmon (eyed egg), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	41.7	>348,320[f]	-	Hamilton and Buhl 1990b
Chinook salmon (alevin), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	41.7	64,690[f]	-	Hamilton and Buhl 1990b

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Chinook salmon (0.31 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	41.7	**16,980**	-	Hamilton and Buhl 1990b
Chinook salmon (0.46 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenite	41.7	**8,150**	15,596	Hamilton and Buhl 1990b
Rainbow trout, *Oncorhynchus mykiss*	S, U	Sodium selenite	330	4,500	-	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	S, U	Sodium selenite	330	4,200	-	Adams 1976
Rainbow trout, *Oncorhynchus mykiss*	S, U	Sodium selenite	272	1,800	-	Hunn et al. 1987
Rainbow trout (alevin), *Oncorhynchus mykiss*	S, U	Sodium selenite	41	118,000	-	Buhl and Hamilton 1991
Rainbow trout (juvenile), *Oncorhynchus mykiss*	S, U	Sodium selenite	41	9,000	-	Buhl and Hamilton 1991
Rainbow trout, *Oncorhynchus mykiss*	F, M	Sodium selenite	30	**12,500**	-	Goettl and Davies 1976
Rainbow trout, *Oncorhynchus mykiss*	F, M	Sodium selenite	135	**8,800**	10,488	Hodson et al. 1980
Brook trout (adult), *Salvelinus fontinalis*	F, M	Selenium dioxide	157	**10,200**	10,200	Cardwell et al. 1976a,b
Arctic grayling (alevin), *Thymallus arcticus*	S, U	Sodium selenite	41	34,732[f]	-	Buhl and Hamilton 1991
Arctic grayling (juvenile), *Thymallus arcticus*	S, U	Sodium selenite	41	**15,675**	15,675	Buhl and Hamilton 1991
Goldfish, *Carassius auratus*	F, M	Selenium dioxide	157	**26,100**	26,100	Cardwell et al. 1976a,b
Common carp, *Cyprinus carpio*	R, U	-	-	**35,000**	35,000	Sato et al. 1980
Golden shiner, *Notemigonus crysoleucas*	F, M	Sodium selenite	72.2	**11,200**	11,200	Hartwell et al. 1989
Fathead minnow, *Pimephales promelas*	S, U	Sodium selenite	312 (13°C)	10,500	-	Adams 1976
Fathead minnow, *Pimephales promelas*	S, U	Sodium selenite	312 (13°C)	11,300	-	Adams 1976

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Fathead minnow, *Pimephales promelas*	S, U	Sodium selenite	303 (20°C)	6,000	-	Adams 1976
Fathead minnow, *Pimephales promelas*	S, U	Sodium selenite	303 (20°C)	7,400	-	Adams 1976
Fathead minnow, *Pimephales promelas*	S, U	Sodium selenite	292 (25°C)	3,400	-	Adams 1976
Fathead minnow, *Pimephales promelas*	S, U	Sodium selenite	292 (25°C)	2,200	-	Adams 1976
Fathead minnow (30 days), *Pimephales promelas*	S, M	Sodium selenite	51.1	1,700	-	Brooke et al. 1985
Fathead minnow (juvenile), *Pimephales promelas*	S, U	Sodium selenite	40	7,760	-	Mayer and Ellersieck 1986
Fathead minnow (fry), *Pimephales promelas*	F, M	Selenium dioxide	157	**2,100**	-	Cardwell et al. 1976a,b
Fathead minnow (juvenile), *Pimephales promelas*	F, M	Selenium dioxide	157	**5,200**	-	Cardwell et al. 1976a,b
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenite	131 (sulfate=24)	**3,670**	--	GLEC 1998
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenite	131 (sulfate=160)	**2,920**	--	GLEC 1998
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenite	145 (sulfate=214)	**3,390**	--	GLEC 1998
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenite	140 (sulfate=870)	**2,380**	-	GLEC 1998
Fathead minnow, *Pimephales promelas*	F, M	Selenious acid	220[d]	**620**	-	Kimball, Manuscript
Fathead minnow, *Pimephales promelas*	F, M	Selenious acid	220[d]	**970**	2,209	Kimball, Manuscript
Colorado squawfish (fry), *Ptychocheilus lucius*	S, U	Sodium selenite	197	**6,398**	-	Hamilton 1995
Colorado squawfish (0.4-1.1 g juvenile), *Ptychocheilus lucius*	S, U	Sodium selenite	197	**16,452**	-	Hamilton 1995
Colorado squawfish (1.7 g juvenile), *Ptychocheilus lucius*	S, U	Sodium selenite	197	**14,624**	-	Hamilton 1995

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Colorado squawfish (larva), *Ptychocheilus lucius*	S, U	Sodium selenite	199	**7,960**	-	Buhl and Hamilton 1996
Colorado squawfish (juvenile), *Ptychocheilus lucius*	S, U	Sodium selenite	199	**17,350**	-	Buhl and Hamilton 1996
Colorado squawfish (0.024-0.047 g), *Ptychocheilus lucius*	S, U	Sodium selenite	144	**20,700**	12,801	Hamilton and Buhl 1997a
Bonytail (fry), *Gila elegans*	S, U	Sodium selenite	197	**8,680**	-	Hamilton 1995
Bonytail (1.1 g juvenile), *Gila elegans*	S, U	Sodium selenite	197	**7,769**	-	Hamilton 1995
Bonytail (2.6 g juvenile), *Gila elegans*	S, U	Sodium selenite	197	**6,855**	-	Hamilton 1995
Bonytail (larva), *Gila elegans*	S, U	Sodium selenite	199	**14,490**	-	Buhl and Hamilton 1996
Bonytail (juvenile), *Gila elegans*	S, U	Sodium selenite	199	**12,870**	9,708	Buhl and Hamilton 1996
Razorback sucker (fry), *Xyrauchen texanus*	S, U	Sodium selenite	197	**6,855**	-	Hamilton 1995
Razorback sucker (0.9 g juvenile), *Xyrauchen texanus*	S, U	Sodium selenite	197	**4,067**	-	Hamilton 1995
Razorback sucker (2.0 g juvenile), *Xyrauchen texanus*	S, U	Sodium selenite	197	**7,312**	-	Hamilton 1995
Razorback sucker (larva), *Xyrauchen texanus*	S, U	Sodium selenite	199	**10,450**	-	Buhl and Hamilton 1996
Razorback sucker (juvenile), *Xyrauchen texanus*	S, U	Sodium selenite	199	**8,520**	-	Buhl and Hamilton 1996
Razorback sucker (0.006-0.042 g), *Xyrauchen texanus*	S, U	Sodium selenite	144	**11,300**	7,679	Hamilton and Buhl 1997a
White sucker, *Catostomus commersoni*	F, M	Sodium selenite	10.2	**29,000**	-	Klaverkamp et al. 1983a
White sucker, *Catostomus commersoni*	F, M	Sodium selenite	18	**31,400**	30,176	Duncan and Klaverkamp 1983

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Flannelmouth sucker (12-13 days), *Catostomus latipinnis*	S, U	Sodium selenite	144	**19,100**	19,100	Hamilton and Buhl 1997b
Striped bass (63 days), *Morone saxatilis*	S, U	Sodium selenite	40	**1,325**	-	Palawski et al. 1985
Striped bass (63 days), *Morone saxatilis*	S, U	Sodium selenite	285	**2,400**	1,783	Palawski et al. 1985
Channel catfish (juvenile), *Ictalurus punctatus*	S, M	Sodium selenite	49.8	16,000	-	Brooke et al. 1985
Channel catfish (juvenile), *Ictalurus punctatus*	S, U	Sodium selenite	41	4,110	-	Mayer and Ellersieck 1986
Channel catfish, *Ictalurus punctatus*	F, M	Selenium dioxide	157	**13,600**	13,600	Cardwell et al. 1976a,b
Flagfish, *Jordanella floridae*	F, M	Selenium dioxide	157	**6,500**	6,500	Cardwell et al. 1976a,b
Mosquitofish, *Gambusia affinis*	S, U	Sodium selenite	45.7	**12,600**	12,600	Reading 1979
Bluegill (juvenile), *Lepomis macrochirus*	S, M	Sodium selenite	50.5	12,000	-	Brooke et al. 1985
Bluegill, *Lepomis macrochirus*	F, M	Selenium dioxide	157	**28,500**	28,500	Cardwell et al. 1976a,b
Yellow perch, *Perca flavescens*	F, M	Sodium selenite	10.2	**11,700**	11,700	Klaverkamp et al. 1983a

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
FRESHWATER SPECIES							
Selenate							
Hydra (adult), *Hydra sp.*	S, M	Sodium selenate	53.6 (sulfate=12)	7300	**25,032**	25,032	Brooke et al. 1985
Leech (adult), *Nephelopsis obscura*	S, M	Sodium selenate	49.3 (sulfate=12)	442000	**1,515,661**	1,515,661	Brooke et al. 1985
Snail, *Aplexa hypnorum*	S, M	Sodium selenate	51.0 (sulfate=12)	193000	**661,816**	661,816	Brooke et al. 1985

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=52)	1967	2,877	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=55)	1864	2,638	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=31)	1078	2,129	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=38)	580	1,018	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=98)	1822	1,844	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=98)	1728	1,748	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=213)	1453	936	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=217)	2812	1,793	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=378)	5553	2,564	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=378)	5481	2,531	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=926)	9157	2,512	--	Brix et al. 2001a,b

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Cladoceran (<24 hr), *Ceriodaphnia dubia*	S, M	Sodium selenate	52 (sulfate=1205)	9311	2,191	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Ceriodaphnia dubia*	F, M	Sodium selenate	127 (sulfate=25)	376	**842**	842	GLEC 1999
Cladoceran, *Daphnia magna*	S, M	Sodium selenate	129.5 (sulfate=163)	5300	**3,990**	--	Dunbar et al. 1983
Cladoceran, *Daphnia magna*	S, M	Sodium selenate	138 (sulfate=22)	1010	**2,435**	--	Boyum 1984
Cladoceran, *Daphnia magna*	S, M	Sodium selenate	48.1 (sulfate=12)	570	**1,955**	--	Brooke et al. 1985
Cladoceran, *Daphnia magna*	S, M	Sodium selenate	45.5 (sulfate=41)	2560	**4,298**	--	Ingersoll et al. 1990
Cladoceran, *Daphnia magna*	S, M	Sodium selenate	136 (sulfate=68)	4070	**5,093**	--	Ingersoll et al. 1990
Cladoceran (<24 hr), *Daphnia magna*	R, M	Sodium selenate	80-100 (sulfate=82)	2840	**3,187**	3,314	Maier et al. 1993
Cladoceran (<24 hr), *Daphnia pulex*	S, M	Sodium selenate	52 (sulfate=54)	10123	14,482	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Daphnia pulex*	S, M	Sodium selenate	52 (sulfate=38)	8126	14,233	--	Brix et al. 2001a,b
Cladoceran (<24 hr), *Daphnia pulex*	F, M	Sodium selenate	147 (sulfate=25)	1528	**3,420**	3,420	GLEC 1999
Cladoceran, *Daphnia pulicaria*	S, M	Sodium selenate	138 (sulfate=22)	246	**593**	593	Boyum 1984
Amphipod (8-12 mm), *Gammarus lacustris*	S, M	Sodium selenate	116 (sulfate=120)	3054	**2,747**	2,747	Brix et al. 2001a,b
Amphipod (adult), *Gammarus pseudolimnaeus*	S, M	Sodium selenate	46.1 (sulfate=12)	75	257	--	Brooke et al. 1985
Amphipod (adult), *Gammarus pseudolimnaeus*	S, M	Sodium selenate	51.0 (sulfate=12)	57	196	--	Brooke 1987

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenate	139 (sulfate=25)	1180	**2,641**	--	GLEC 1998
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenate	132 (sulfate=125)	2870	**2,521**	--	GLEC 1998
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenate	137 (sulfate=367)	3710	**1,743**	--	GLEC 1998
Amphipod, *Gammarus pseudolimnaeus*	F, M	Sodium selenate	134 (sulfate=635)	3270	**1,167**	--	GLEC 1998
Amphipod (adult), *Gammarus pseudolimnaeus*	F, M	Sodium selenate	131 (sulfate=25)	2191	**4,904**	2,315	GLEC 1999
Amphipod, *Hyalella azteca*	F, U	Sodium selenate	336.8 (sulfate NA)	760	--	--	Adams 1976
Amphipod (2 mm length), *Hyalella azteca*	R, M	Sodium selenate	133 (sulfate=13)	1031	3,375	-	Brasher and Ogle 1993
Amphipod (7-10 days), *Hyalella azteca*	S, M	Sodium selenate	52 (sulfate=55)	1424	2,021	--	Brix et al. 2001a,b
Amphipod, *Hyalella azteca*	F, M	Sodium selenate	143 (sulfate=40)	2480	**4,224**	--	GLEC 1998
Amphipod, *Hyalella azteca*	F, M	Sodium selenate	132 (sulfate=125)	1350	**1,186**	--	GLEC 1998
Amphipod, *Hyalella azteca*	F, M	Sodium selenate	137 (sulfate=367)	1540	**723**	--	GLEC 1998
Amphipod, *Hyalella azteca*	F, M	Sodium selenate	133 (sulfate=822)	3580	**1,052**	1,397	GLEC 1998
Midge (4th instar), *Chironomus decorus*	R, M	Sodium selenate	85 (sulfate=27)	23700	**50,727**	50,727	Maier and Knight 1993
Midge (3rd instar), *Paratanytarsus parthenogeneticus*	S, M	Sodium selenate	49.4 (sulfate=12)	20000	**68,582**	68,582	Brooke et al. 1985
Coho salmon (0.5 g), *Oncorhynchus kisutch*	S, U	Sodium selenate	211 (sulfate=185)	32500	**22,730**	--	Hamilton and Buhl 1990b

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Coho salmon (1.7 g), *Oncorhynchus kisutch*	S, U	Sodium selenate	333 (sulfate=291)	39000	**20,963**	--	Hamilton and Buhl 1990b
Coho salmon (alevin), *Oncorhynchus kisutch*	S, U	Sodium selenate	41 (sulfate=41)	158,422[f]	265,990[f]	--	Buhl and Hamilton 1991
Coho salmon (juvenile), *Oncorhynchus kisutch*	S, U	Sodium selenate	41 (sulfate=41)	30932	**51,935**	29,141	Buhl and Hamilton 1991
Chinook salmon (0.7 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	211 (sulfate=185)	121000	**84,626**	--	Hamilton and Buhl 1990b
Chinook salmon (0.5 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	211 (sulfate=185)	100000	**69,939**	--	Hamilton and Buhl 1990b
Chinook salmon (1.6 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	333 (sulfate=291)	180000	**96,752**	--	Hamilton and Buhl 1990b
Chinook salmon (1.6 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	333 (sulfate=291)	134000	**72,026**	--	Hamilton and Buhl 1990b
Chinook salmon (eyed egg), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	41.7 (sulfate=47)	>552,000[f]	>856,083[f]	--	Hamilton and Buhl 1990b
Chinook salmon (alevin), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	41.7 (sulfate=47)	>176,640[f]	>273,947[f]	--	Hamilton and Buhl 1990b
Chinook salmon (0.31 g), *Oncorhynchus tshawytscha*	S, U	Sodium selenate	41.7 (sulfate=47)	62900	**97,550**	83,353	Hamilton and Buhl 1990b
Rainbow trout (juvenile), *Oncorhynchus mykiss*	S, M	Sodium selenate	51.0 (sulfate=12)	24000	**82,298**	--	Brooke et al. 1985

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Rainbow trout (alevin), *Oncorhynchus mykiss*	S, U	Sodium selenate	41 (sulfate=41)	196460	329,856[f]	--	Buhl and Hamilton 1991
Rainbow trout, *Oncorhynchus mykiss*	F, M	Sodium selenate	45 (sulfate=12)	47000	161,168[f]	--	Spehar 1986
Rainbow trout (juvenile), *Oncorhynchus mykiss*	S, U	Sodium selenate	41 (sulfate=41)	13501	**22,668**	43,192	Buhl and Hamilton 1991
Arctic grayling (alevin), *Thymallus arcticus*	S, U	Sodium selenate	41 (sulfate=41)	41800	**70,182**	--	Buhl and Hamilton 1991
Arctic grayling (juvenile), *Thymallus arcticus*	S, U	Sodium selenate	41 (sulfate=41)	75240	**126,328**	94,159	Buhl and Hamilton 1991
Fathead minnow, *Pimephales promeles*	S, U	Sodium selenate	323 (sulfate NA)	11800	--	--	Adams 1976
Fathead minnow, *Pimephales promeles*	S, U	Sodium selenate	323 (sulfate NA)	11000	--	--	Adams 1976
Fathead minnow, *Pimephales promeles*	S, U	Sodium selenate	323 (sulfate NA)	12500	--	--	Adams 1976
Fathead minnow (juvenile), *Pimephales promelas*	S, M	Sodium selenate	47.9 (sulfate =12)	2300	7,887	--	Brooke et al. 1985
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenate	46 (sulfate =12)	5500	**18,860**	--	Spehar 1986
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenate	136 (sulfate=24)	6210	**14,236**	--	GLEC 1998
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenate	127 (sulfate=160)	10800	**8,218**	--	GLEC 1998
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenate	131 (sulfate=474)	18000	**7,286**	--	GLEC 1998

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Fathead minnow, *Pimephales promelas*	F, M	Sodium selenate	147 (sulfate=906)	42100	**11,695**	11,346	GLEC 1998
Colorado squawfish (fry), *Ptychocheilus lucius*	S, U	Sodium selenate	196 (sulfate=164)	27588	**20,694**	--	Hamilton 1995
Colorado squawfish (0.4-1.1 g juvenile), *Ptychocheilus lucius*	S, U	Sodium selenate	196 (sulfate=164)	119548	89,676[f]	--	Hamilton 1995
Colorado squawfish (1.7 g juvenile), *Ptychocheilus lucius*	S, U	Sodium selenate	196 (sulfate=164)	138358	103,786[f]	--	Hamilton 1995
Colorado squawfish (larva), *Ptychocheilus lucius*	S, U	Sodium selenate	199 (sulfate=174)	13580	**9,842**	--	Buhl and Hamilton 1996
Colorado squawfish (0.024-0.047 g), *Ptychocheilus lucius*	S, U	Sodium selenate	144 (sulfate=97)	88000	89,572[f]	--	Hamilton and Buhl 1997a
Colorado squawfish (juvenile), *Ptychocheilus lucius*	S, U	Sodium selenate	199 (sulfate=174)	42780	**31,005**	18,484	Buhl and Hamilton 1996
Bonytail (fry), *Gila elegans*	S, U	Sodium selenate	196 (sulfate=164)	22990	17,245[f]	--	Hamilton 1995
Bonytail (1.1 g juvenile), *Gila elegans*	S, U	Sodium selenate	196 (sulfate=164)	102828	77,134[f]	--	Hamilton 1995
Bonytail (2.6 g juvenile), *Gila elegans*	S, U	Sodium selenate	196 (sulfate=164)	90706	68,041[f]	--	Hamilton 1995
Bonytail (juvenile), *Gila elegans*	S, U	Sodium selenate	199 (sulfate=174)	24010	17,401[f]	--	Buhl and Hamilton 1996

Table 1a. Acute Toxicity of Selenium to Freshwater Animals (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	LC50 or EC50 (µg/L)[b]	LC50 or EC50 Adj. To Sulfate = 100 (µg/L)	Species Mean Acute Value at Sulfate = 100 (µg/L)	Reference
Bonytail (larva), *Gila elegans*	S, U	Sodium selenate	199 (sulfate=174)	14570	**10,560**	10,560	Buhl and Hamilton 1996
Razorback sucker (fry), *Xyrauchen texanus*	S, U	Sodium selenate	196 (sulfate=164)	20064	**15,051**	--	Hamilton 1995
Razorback sucker (0.9 g juvenile), *Xyrauchen texanus*	S, U	Sodium selenate	196 (sulfate=164)	15048	**11,288**	- -	Hamilton 1995
Razorback sucker (2.0 g juvenile), *Xyrauchen texanus*	S, U	Sodium selenate	196 (sulfate=164)	10450	**7,839**	--	Hamilton 1995
Razorback sucker (larva), *Xyrauchen texanus*	S, U	Sodium selenate	199 (sulfate=174)	13910	**10,081**	--	Buhl and Hamilton 1996
Razorback sucker (juvenile), *Xyrauchen texanus*	S, U	Sodium selenate	199 (sulfate=174)	7620	**5,523**	--	Buhl and Hamilton 1996
Razorback sucker (0.006-0.042 g), *Xyrauchen texanus*	S, U	Sodium selenate	144 (sulfate=97)	15900	**16,184**	10,309	Hamilton and Buhl 1997a
Flannelmouth sucker (12-13 days), *Catostomus latipinnis*	S, U	Sodium selenate	144 (sulfate=97)	26900	**27,380**	27,380	Hamilton and Buhl 1997b
Channel catfish (juvenile), *Ictalurus punctatus*	S, M	Sodium selenate	51.0 (sulfate=12)	66000	**226,320**	226,320	Brooke et al. 1985
Bluegill (juvenile), *Lepomis macrochirus*	S, M	Sodium selenate	50.4 (sulfate=12)	63000	**216,033**	216,033	Brooke et al. 1985

[a] S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.
[b] Concentration of selenium, not the chemical. **Note:** The values underlined in this column were used to calculate the SMAV for the respective species.
[c] Reported by Barrows et al. (1980) in work performed in the same laboratory under the same contract.
[d] From Smith et al. (1976).
[e] Calculated from regression equation.
[f] Not used in calculation of Species Mean Acute Value because data are available for a more sensitive life stage.

Table 1b. Acute Toxicity of Selenium to Saltwater Animals

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
SALTWATER SPECIES						
Selenite						
Blue mussel (embryo), *Mytilus edulis*	S, U	Selenium oxide	33.79	**>10,000**	>10,000	Martin et al. 1981
Bay scallop (juvenile), *Argopecten irradians*	R, U	Sodium selenite	25	**255**	255	Nelson et al. 1988
Pacific oyster (embryo), *Crassostrea gigas*	S, U	Selenium oxide	33.79	**>10,000**	-	Glickstein 1978; Martin et al. 1981
Pacific oyster (embryo), *Crassostrea gigas*	S, U	Sodium selenite	33.79	**>10,000**	>10,000	Glickstein 1978
Surf clam (juvenile), *Spisula solidissima*	R, U	Sodium selenite	25	**1,900**	1,900	Nelson et al. 1988
Copepod (adult), *Acartia clausi*	S, U	Selenious acid	30	**2,110**	2,110	Lussier 1986
Copepod (adult), *Acartia tonsa*	S, U	Selenious acid	30	**839**	839	Lussier 1986
Mysid (juvenile), *Americamysis bahia*	S, U	Selenious acid	-	600	-	U.S. EPA 1978
Mysid (juvenile), *Americamysis bahia*	F, M	Selenious acid	15-20	**1,500**	1,500	Ward et al. 1981
Brown shrimp (juvenile), *Penaeus aztecus*	S, U	Sodium selenite	30	**1,200**	1,200	Ward et al. 1981
Dungeness crab (zoea 1arva), *Cancer magister*	S, U	Selenium oxide	33.79	**1,040**	1,040	Glickstein 1978
Blue crab (juvenile), *Callinectes sapidus*	S, U	Sodium selenite	30	**4,600**	4,600	Ward et al. 1981

Table 1b. Acute Toxicity of Selenium to Saltwater Animals (continued).

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Haddock (larva), *Melanogrammus aeglefinus*	S, U	Selenious acid	30	**599**	599	Cardin 1986
Sheepshead minnow (juvenile), *Cyrinodon variegatus*	S, U	Selenious acid	-	6,700	-	Heitmuller et al. 1981
Sheepshead minnow (juvenile), *Cyrinodon variegatus*	F, M	Sodium selenite	30	**7,400**	7,400	Ward et al. 1981
Atlantic silverside (juvenile), *Menidia menidia*	S, U	Selenious acid	30	**9,725**	9,725	Cardin 1986
Fourspine stickleback (adult), *Apeltes quadracus*	S, U	Selenious acid	30	**17,350**	17,350	Cardin 1986
Striped bass, *Morone saxatilis*	S, U	Sodium selenite	1	**1,550**	-	Palawski et al. 1985
Striped bass (24 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	**3,400**	-	Chapman 1992
Striped bass (25 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	**3,300**	-	Chapman 1992
Striped bass (31 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	**3,800**	-	Chapman 1992
Striped bass (32 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	**3,900**	3,036	Chapman 1992
Pinfish (juvenile), *Lagodon rhomboides*	S, U	Sodium selenite	30	**4,400**	4,400	Ward et al. 1981
Summer flounder (embryo), *Paralichthys dentatus*	S, U	Selenious acid	30.2	**3,497**	3,497	Cardin 1986
Winter flounder (larva), *Pseudopleuronectes americanus*	S, U	Selenious acid	30	**14,240**	-	Cardin 1986
Winter flounder (larva), *Pseudopleuronectes americanus*	S, U	Selenious acid	28	**15,070**	14,649	Cardin 1986

Table 1b. Acute Toxicity of Selenium to Saltwater Animals (continued).

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
			Selenate			
Striped bass (24 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	26,300[c]	-	Chapman 1992
Striped bass (25 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	23,700[c]	-	Chapman 1992
Striped bass (31 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	26,300[c]	-	Chapman 1992
Striped bass (32 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	29,000[c]	-	Chapman 1992
Striped bass (juvenile), *Morone saxatilis*	F, M	Sodium selenate	6.0-6.5	85,840[c]	-	Klauda 1985a,b
Striped bass (prolarvae), *Morone saxatilis*	F, M	Sodium selenate	3.5-4.2	**9,790**	9,790	Klauda 1985a,b

[a] S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.
[b] Concentration of selenium, not the chemical. **Note:** The values underlined in this column were used to calculate the SMAV for the respective species.
[c] Not used in calculation of Species Mean Acute Value because data are available for a more sensitive life stage.

Table 2a. Ranked Freshwater Genus Mean Acute Values

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		FRESHWATER SPECIES		
		Selenite		
28	203,000	Leech, *Nephelopsis obscura*	203,000	1
27	42,500	Midge, *Tanytarsus dissimilis*	42,500	1
26	35,356	Midge, *Chironomus decorus*	48,200	1
		Midge, *Chironomus plumosus*	25,934	2
25	35,000	Common carp, *Cyprinus carpio*	35,000	1
24	34,914	Snail, *Aplexa hypnorum*	34,914	2
23	28,500	Bluegill, *Lepomis macrochirus*	28,500	1
22	26,100	Goldfish, *Carassius auratus*	26,100	1
21	24,100	Snail, *Physa sp.*	24,100	1
20	24,008	White sucker, *Catostomus commersoni*	30,176	2
		Flannelmouth sucker *Catostomus latipinnis*	19,100	1
19	15,675	Arctic grayling *Thymallus arcticus*	15,675	1
18	13,600	Channel catfish, *Ictalurus punctatus*	13,600	1
17	12,801	Colorado squawfish, *Ptychocheilus lucias*	12,801	6
16	12,600	Mosquitofish, *Gambusia affinis*	12,600	1
15	11,700	Yellow perch, *Perca flavescens*	11,700	1
14	11,200	Golden shiner, *Notemigonus crysoleucas*	11,200	1
13	10,580	Chinook salmon, *Oncorhynchus tshawytscha*	15,596	6

Table 2a. Ranked Freshwater Genus Mean Acute Values (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		Coho salmon, *Oncorhynchus kisutch*	7,240	3
		Rainbow trout, *Oncorhynchus mykiss*	10,488	2
12	10,200	Brook trout *Salvelinus fontinalis*	10,200	1
11	9,708	Bonytail *Gilas elegans*	9,708	5
10	7,710	Worm, *Tubifex tubifex*	7,710	1
9	7,679	Razorback sucker, *Xyrauchen texanus*	7,679	6
8	6,500	Flagfish, *Jordanella floridae*	6,500	1
7	3,489	Amphipod, *Gammarus pseudolimnaeus*	3,489	5
6	2,209	Fathead minnow, *Pimephales promelas*	2,209	8
5	1,783	Striped bass, *Morone saxatilis*	1,783	2
4	1,700	Hydra, *Hydra sp.*	1,700	1
3	1,341	Cladoceran, *Daphnia magna*	905.3	11
		Cladoceran, *Daphnia pulex*	1,987	1
2	<515.3	Cladoceran, *Ceriodaphnia affinis*	<603.6	4
		Cladoceran, *Ceriodaphnia dubia*	440	1
1	461.4	Amphipod, *Hyalella azteca*	461.4	5

Table 2a. Ranked Freshwater Genus Mean Acute Values (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		Selenate (at sulfate = 100 mg/L)		
18	1,515,661	Leech, *Nephelopsis obscura*	1,515,661	1
17	661,816	Snail, *Aplexa hypnorum*	661,816	1
16	226,320	Channel catfish, *Ictalurus punctatus*	226,320	1
15	216,033	Bluegill, *Lepomis macrochirus*	216,033	1
14	94,159	Arctic grayling, *Thymallus arcticus*	94,159	2
13	68,582	Midge, *Paratanytarsus parthenogeneticus*	68,582	1
12	50,727	Midge, *Chironomus decorus*	50,727	1
11	47,164	Chinook salmon, *Oncorhynchus tshawytscha*	83,353	5
		Coho salmon, *Oncorhynchus kisutch*	29,141	3
		Rainbow trout, *Oncorhynchus mykiss*	43,192	2
10	27,380	Flannelmouth sucker *Catostomus latipinnis*	27,380	1
9	25,032	Hydra, *Hydra sp.*	25,032	1
8	18,484	Colorado squawfish, *Ptychocheilus lucius*	18,484	3
7	11,346	Fathead minnow, *Pimephales promelas*	11,346	5
6	10,560	Bonytail, *Gila elegans*	10,560	1
5	10,309	Razorback sucker, *Xyrauchen texanus*	10,309	6
4	2,522	Amphipod, *Gammarus lacustris*	2,747	1
		Amphipod, *Gammarus pseudolimnaeus*	2,315	5

Table 2a. Ranked Freshwater Genus Mean Acute Values (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
3	1,887	Cladoceran, *Daphnia magna*	3,314	6
		Cladoceran, *Daphnia pulex*	3,420	1
		Cladoceran, *Daphnia pulicaria*	593	1
2	1,397	Amphipod, *Hyalella azteca*	1,397	4
1	842	Cladoceran, *Ceriodaphnia dubia*	842	1

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value. Inclusion of "greater than" and "less than" values does not necessarily imply a true ranking, but does allow use of all genera for which data are available so that the Final Acute Value is not unnecessarily lowered.
[b] From Table 1a.

Table 2b. Ranked Saltwater Genus Mean Acute Values

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		SALTWATER SPECIES		
		Selenite		
17	17,350	Fourspine stickleback, *Apeltes quadracus*	17,350	1
16	14,649	Winter flounder, *Pseudopleuronectes americanus*	14,649	2
15	>10,000	Blue mussel, *Mytilus edulis*	>10,000	1
14	>10,000	Pacific oyster, *Crassostrea gigas*	>10,000	2
13	9,725	Atlantic silverside, *Menidia menidia*	9,725	1
12	7,400	Sheepshead minnow, *Cyprinodon variegatus*	7,400	1
11	4,600	Blue crab, *Callinectes sapidus*	4,600	1
10	4,400	Pinfish, *Lagodon rhomboides*	4,400	1
9	3,497	Summer flounder, *Paralichthys dentatus*	3,497	1
8	3,036	Striped bass, *Morone saxatilis*	3,036	5
7	1,900	Surf clam, *Spisula solidissima*	1,900	1
6	1,500	Mysid, *Americamysis bahia*	1,500	1
5	1,331	Copepod, *Acartia clausi*	2,110	1
		Copepod, *Acartia tonsa*	839	1
4	1,200	Brown shrimp, *Penaeus aztecus*	1,200	1
3	1,040	Dungeness crab, *Cancer magister*	1,040	1
2	599	Haddock, *Melanogrammus aeglefinus*	599	1

Table 2b. Ranked Saltwater Genus Mean Acute Values

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
1	255	Bay scallop, *Argopecten irradians*	255	1
		Selenate		
1	9,790	Striped bass, *Morone saxatilis*	9,790	1

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value. Inclusion of "greater than" and "less than" values does not necessarily imply a true ranking, but does allow use of all genera for which data are available so that the Final Acute Value is not unnecessarily lowered.
[b] From Table 1b.

Selenite

Fresh Water

Final Acute Value = 514.9 µg/L

Criterion Maximum Concentration = (514.9 µg/L) ÷ 2 = 257 µg/L

Salt Water

Final Acute Value = 253.4 µg/L

Criterion Maximum Concentration = (253.4 µg/L) ÷ 2 = 127 µg/L

Selenate

Fresh Water

Final Acute Value = 834.4 µg/L (calculated at a sulfate level of 100 mg/L from GMAVs)

Criterion Maximum Concentration = (834.4 µg/L) ÷ 2 = 417 µg/L (at a sulfate level of 100 mg/L)

Pooled Slope = 0.5812 (see Appendix A)

ln (Criterion Maximum Intercept) = ln(417.2) - [slope x ln(100)]

= 6.0335 - (0.5812 x 4.605) = 3.357

Criterion Maximum Concentration for Selenate (at a sulfate level of 100 mg/L) = $e^{(0.5812[\ln(\text{sulfate})]+3.357)}$

Table 3a. Ratios of Freshwater Species Mean Acute Values for Selenite and Selenate.

Selenite Sensitivity Rank from Table 2a[a]	Species	Selenite Species Mean Acute Value (µg/L)[b]	Selenate Species Mean Acute Value at Sulfate = 100 (µg/L)[b]	Ratio
	FRESHWATER SPECIES			
28	Leech, *Nephelopsis obscura*	203,000	1,515,661	0.134
27	Midge, *Tanytarsus dissimilis*	42,500	NA[c]	NA
26	Midge, *Chironomus decorus*	48,200	50,727	0.95
	Midge, *Chironomus plumosus*	25,934	NA	NA
25	Common carp, *Cyprinus carpio*	35,000	NA	NA
24	Snail, *Aplexa hypnorum*	34,914	616,816	0.057
23	Bluegill, *Lepomis macrochirus*	28,500	216,033	0.132
22	Goldfish, *Carassius auratus*	26,100	NA	NA
21	Snail, *Physa sp.*	24,100	NA	NA
20	White sucker, *Catostomus commersoni*	30,176	NA	NA
	Flannelmouth sucker *Catostomus latipinnis*	19,100	27,380	0.698
19	Arctic grayling *Thymallus articus*	15,675	94,159	0.166
18	Channel catfish, *Ictalurus punctatus*	13,600	226,320	0.06
17	Colorado squawfish, *Ptychocheilus lucias*	12,801	18,484	0.693
16	Mosquitofish, *Gambusia affinis*	12,600	NA	NA
15	Yellow perch, *Perca flavescens*	11,700	NA	NA
14	Golden shiner, *Notoemigonus crysoleucas*	11,200	NA	NA

Table 3a. Ratios of Freshwater Species Mean Acute Values for Selenite and Selenate (continued).

Selenite Sensitivity Rank from Table 2a[a]	Species	Selenite Species Mean Acute Value (µg/L)[b]	Selenate Species Mean Acute Value at Sulfate = 100 (µg/L)[b]	Ratio
13	Chinook salmon, *Oncorhynchus tshawytscha*	15,596	83,353	0.187
	Coho salmon, *Oncorhynchus kisutch*	7,240	29,141	0.248
	Rainbow trout, *Oncorhynchus mykiss*	10,488	43,192	0.243
12	Brook trout *Salvelinus fontinalis*	10,200	NA	NA
11	Bonytail *Gilas elegans*	9,708	10,560	0.919
10	Worm, *Tubifex tubifex*	7,710	NA	NA
9	Razorback sucker, *Xyrauchen texanus*	7,679	10,309	0.745
8	Flagfish, *Jordanella floridae*	6,500	NA	NA
7	Amphipod, *Gammarus pseudolimnaeus*	3,489	2,315	1.507
6	Fathead minnow, *Pimephales promelas*	2,209	11,346	0.195
5	Striped bass, *Morone saxatilis*	1,783	NA	NA
4	Hydra, *Hydra sp.*	1,700	25,032	0.068
3	Cladoceran, *Daphnia magna*	905.3	3,314	0.273
	Cladoceran, *Daphnia pulex*	1,987	3,420	0.581
2	Cladoceran, *Ceriodaphnia affinis*	<603.6	NA	NA
	Cladoceran, *Ceriodaphnia dubia*	440	842	0.523
1	Amphipod, *Hyalella azteca*	461.4	1,397	0.33

[a] Ranked from most resistant to most sensitive based on selenite Genus Mean Acute Value (from Table 2a).
[b] From Table 1a.
[c] NA = Not Available

Table 3b. Ratios of Saltwater Species Mean Acute Values for Selenite and Selenate.

Sensitivity Rank from Table 2b[a]	Species	Selenite Species Mean Acute Value (µg/L)[b]	Selenate Species Mean Acute Value (µg/L)[b]	Ratio
	SALTWATER SPECIES			
8	Striped bass, *Morone saxatilis*	3,036	9,790	0.31

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value (from Table 2b).
[b] From Table 1b.

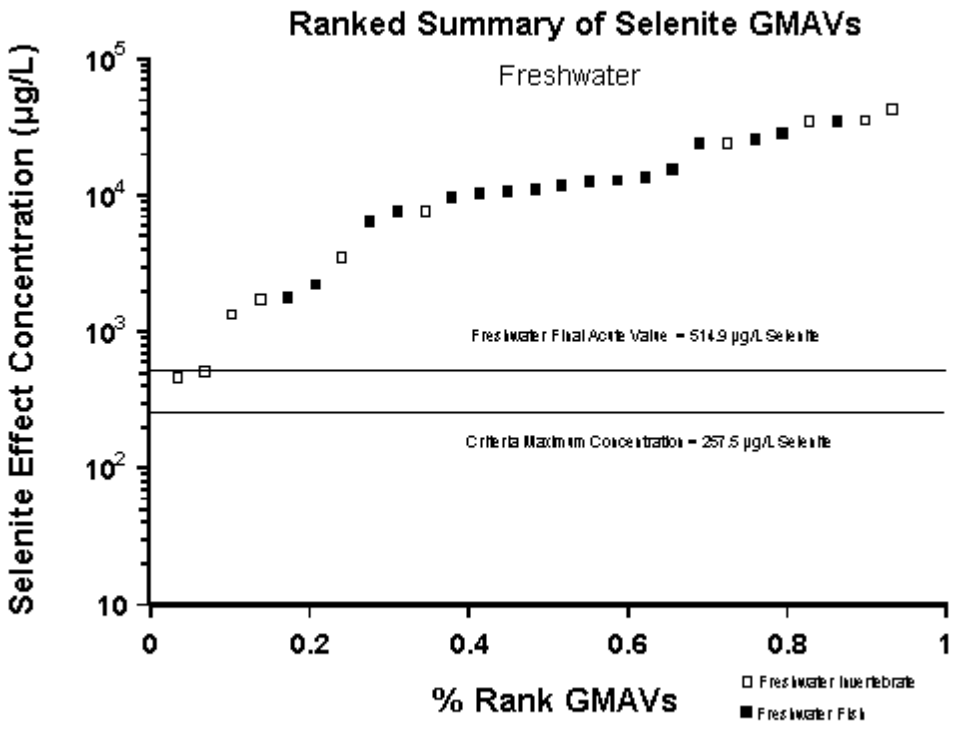

Figure 1. Ranked summary of selenite GMAVs (freshwater).

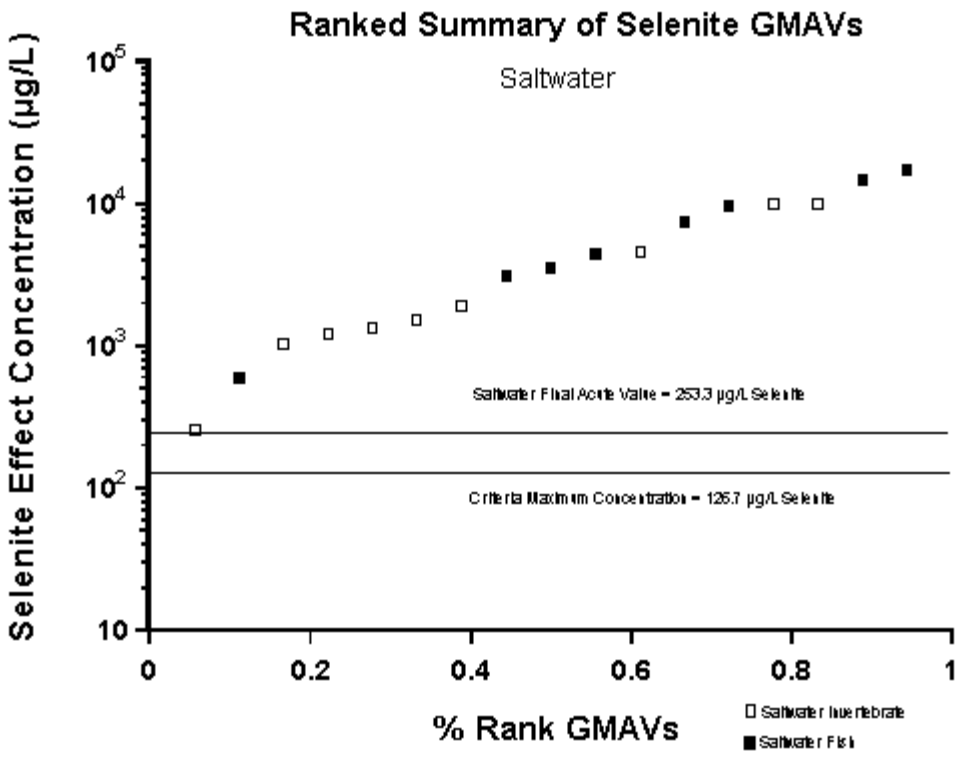

Figure 2. Ranked summary of selenate GMAVs (saltwater).

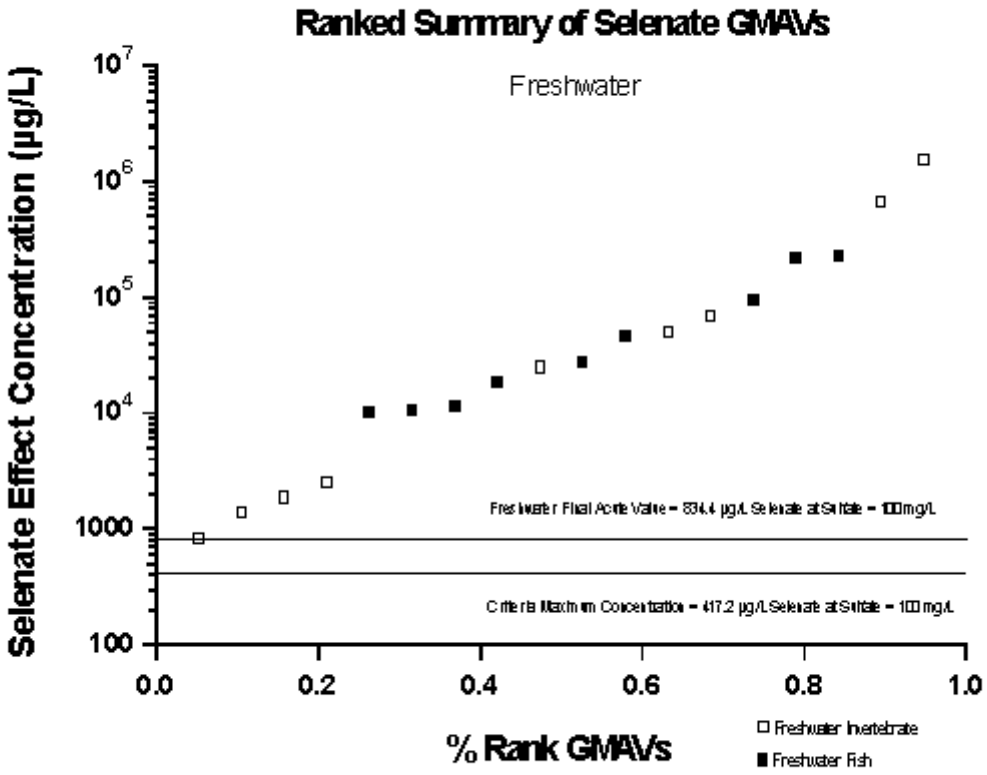

Figure 3. Ranked summary of selenate GMAVs (freshwater) at a sulfate level of 100 mg/L.

Review and Analysis of Chronic Data

Since the issuance of the 1987 chronic criterion of 5 µg/L, considerable information has come forth regarding the route of exposure of selenium to aquatic organisms. Studies have shown that diet is the primary route of exposure that controls chronic toxicity to fish, the group considered to be the most sensitive to chronic selenium exposure (Coyle et al. 1993; Hamilton et al. 1990; Hermanutz et al. 1996). Chronic tests in which test organisms were exposed to selenium only through water and which have measured selenium in the tissue of the test species have produced questionably low chronic values based on the tissue concentrations. Some of these water-only exposures have required aqueous concentrations of selenium of greater than 300 µg/L to attain body burdens sufficient to achieve a chronic response that would have been reached in the real world at aqueous concentrations approximately 30 times lower (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978).

Because diet controls selenium chronic toxicity in the environment and water-only exposures require unrealistic aqueous concentrations in order to elicit a chronic response, only studies in which test organisms were exposed to selenium in their diet alone or in their diet and water were considered in the derivation of a chronic value. To be able to use the chronic study results, the measurements had to include selenium in the test species tissue. Both laboratory and field studies were considered in the review process. Chronic studies reviewed were obtained through a literature search extending back to the last revision review, from information supplied to U.S. EPA through the Notice of Data Availability, and using the references cited in previous selenium criteria documents.

Selection of Medium for Expressing Chronic Criterion

Whole-body tissue concentration of selenium on a dry weight basis, for species eliciting the chronic response, was selected as the medium from which to base the chronic criterion value. As discussed above, a water-based criterion is not appropriate for selenium because diet is the most important route of exposure for chronic toxicity. The option of basing the chronic criterion on the concentration of selenium in prey species (that is, in the diet of the target species), was considered inappropriate for two reasons: 1) the concentration of selenium in the diet is an indirect measure of effects observed in the test species and is dependent on feeding behavior of the target species, and 2) selection of what organism to sample to assess attainment of a criterion based on diet is problematic in the implementation of such a criterion. Sediment has also been proposed as a medium upon which to base the selenium chronic criterion (Canton and Van Derveer 1997; Van Derveer and Canton 1997), but because of the patchiness

of selenium in sediment and an insufficient amount of data to support a causal link between concentrations of selenium in sediment and chronic effects observed in fish (see Hamilton and Lemly 1999, for a review), a sediment-based criterion was rejected.

Besides being a direct link to chronic endpoints, a tissue-based criterion has the positive attributes of integrating many site-specific factors, such as chemical speciation and rates of transformation, large variations in temporal concentrations in water, types of organisms constituting the food chain, and rates of exchange between water, sediment, and organisms (Hamilton, in preparation; U.S. EPA 1998). Whole-body tissue was selected over specific tissue types, such as ovary, liver, kidney or muscle because of practical reasons of sampling and because a sufficient data base containing chronic effects based on whole-body tissue is present in the literature. Ovaries may be the best tissue to link selenium to reproductive effects because of its role in the maternal transfer of selenium to eggs, and embryo-larval development being one of the most sensitive endpoint for chronic effects. However, ovarian tissue is also only available seasonally and sometimes difficult to extract in quantities sufficient for analysis, especially in smaller fish species. Whole-body larval tissue is also not practical due to sampling and seasonal constraints.

To increase the number of studies in which chronic effects could be compared with selenium concentrations in whole-body tissue, the relationships between selenium concentrations in whole-body and selenium concentrations in ovary, liver, and muscle tissues were estimated. Data from 4 dietary exposure studies that sampled whole-body as well as muscles, ovary, or liver allowed the projection of whole-body concentrations as a function of concentrations in these individual tissues. It was not possible to estimate such relationship for kidneys and carcass because of insufficient data. One species (bluegill sunfish) comprised over 90 percent of the data evaluated for these relationships.

Median concentrations of selenium in the whole-body were projected as a linear function of selenium concentrations in ovaries and liver, or as an exponential function of the natural logarithm of selenium concentrations in muscles (Figure 4; Appendix H). When selenium concentration in more than one organ or tissue was available, muscle tissue was used preferentially for converting into an equivalent whole-body value. Where appropriate, whole-body selenium concentrations were estimated from selenium concentrations in muscle, ovary and liver according to the following equations (see Appendix H for details on statistical analyses):

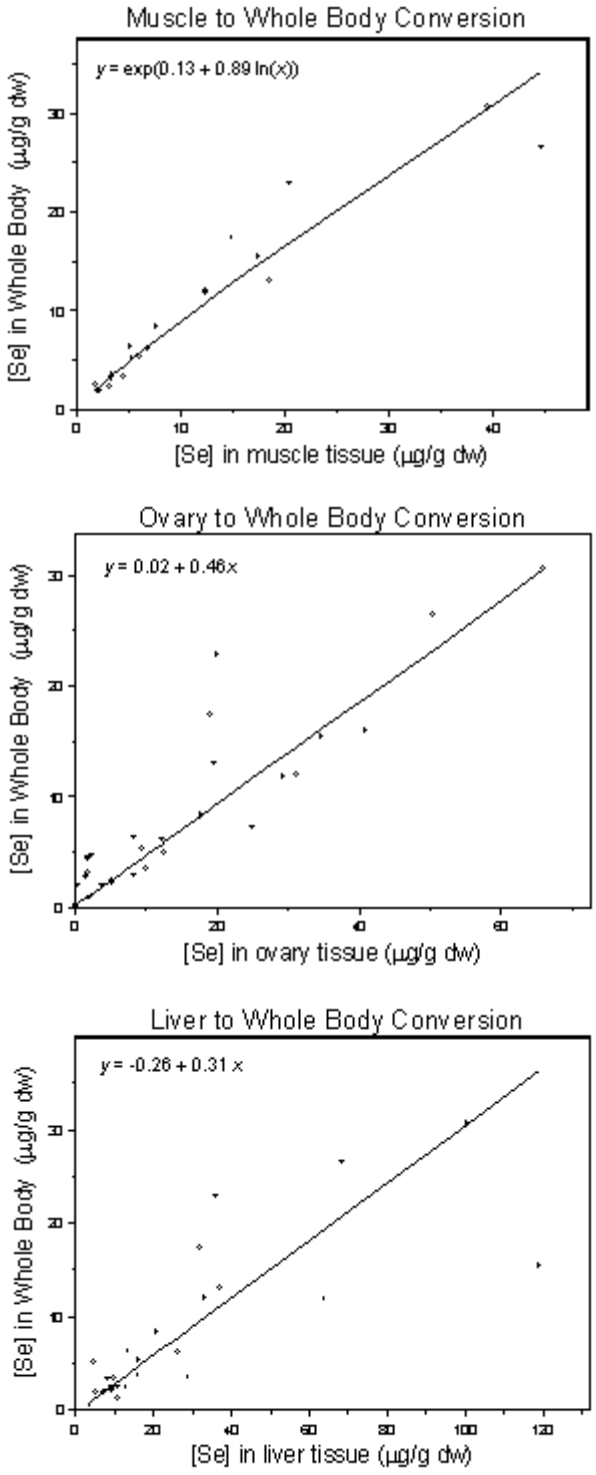

Figure 4. The quantile regression curves project median selenium concentrations in the whole body of bluegill, largemouth bass, tilapia and carp as a function of selenium concentrations in their tissues. Most data are from bluegill. Estimates of model parameters minimize the sum of weighted absolute deviations (see Appendix H for details about statistical analyses).

$$[Se_{whole\text{-}body}] = \exp(0.1331 + (0.8937 \times \ln[Se_{muscle}])) \quad \text{(I)}$$

$$[Se_{whole\text{-}body}] = 0.0173 + (0.4634 \times [Se_{ovary}]) \quad \text{(II)}$$

$$[Se_{whole\text{-}body}] = -0.2609 + (0.3071 \times [Se_{liver}]) \quad \text{(III)}$$

Chronic studies that reported selenium concentrations in tissues based on wet weight were converted to dry weight using a moisture content of 0.80 (U.S. EPA 1985b), unless specified otherwise. Note that because conversion from wet to dry weight and from tissue to whole-body selenium concentration can increase uncertainty in the estimate, site-data analysts should develop their own conversion factors whenever possible to improve accuracy. The basis for such factors can be obtained from local historical data or from newly acquired data specific for that site and species.

Calculation of Chronic Values

In aquatic toxicity tests, chronic values have usually been defined as the geometric mean of the highest concentration of a toxic substance at which no adverse effect is observed (highest no observed adverse effect concentration, NOAEC) and the lowest concentration of the toxic substance that causes an adverse effect (lowest observed adverse effect concentration, LOAEC). The significance of observed effects is determined by statistical tests comparing responses of organisms exposed to natural concentrations of the toxic substance (control) against responses of organisms exposed to elevated concentrations. Analysis of variance is the most common test employed for such comparisons. This approach however, has its limitations. Since neither NOAEC or LOAEC are known in advance and the number of concentrations that can be tested is constrained by logistic and financial resources, observed effects of elevated concentrations may not permit accurate estimates of chronic values. For instance, if all elevated concentrations had high adverse effects or if the difference in concentrations between two significantly different treatments was large, it would not be possible to define either the NOAEC or LOAEC with precision. Furthermore, as the concentration of some substances (e.g., selenium) naturally varies among ecosystems, a concentration that is above the normal range at one site, maybe within the normal range at a different location. In this approach to calculate chronic values, natural variation in concentrations of a substance implies that controls are site specific, and thus multiple tests are needed to define the chronic value at different locations.

An alternative approach to calculate chronic values focuses on the use of regression analysis to define the dose-response relationship. With a regression equation, which defines the level of adverse effects as a function of increasing concentrations of the toxic substance, it is possible to determine the concentration that causes a relatively small effect, for example a 5 to 30 percent reduction in response. A reduction of 20 percent in the response observed at control (EC_{20}) was used as the chronic value because it represents a low level of effect that is generally significantly different from the control (U.S. EPA 1999). Smaller reductions in growth, survival, or other endpoints only rarely can be detected statistically. Effect concentrations associated with such small reductions have wide uncertainty bands, making them unreliable for criteria derivation. Adverse effects are generally modeled as a sigmoid function of increasing concentrations of the toxic substance (Figure 5).

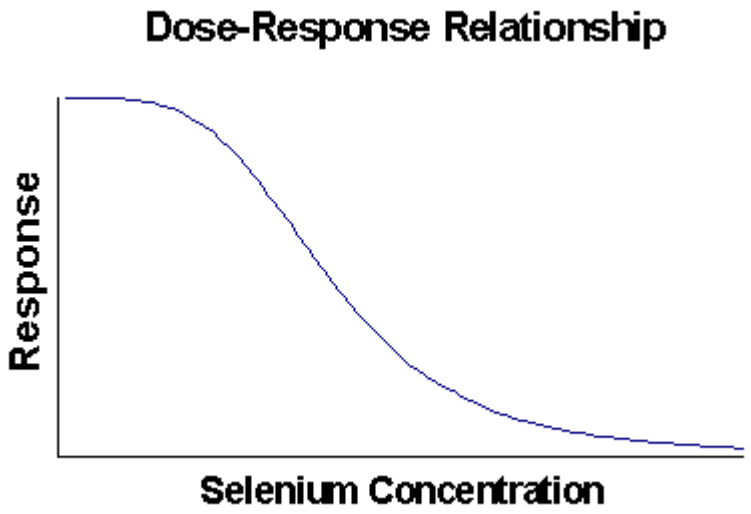

Figure 5. Reductions in survival, growth or other responses of organisms are often modeled as a sigmoid function of increasing concentrations of selenium, or any other toxic substance.

A logistic regression was used to model negative effects of increasing concentrations of selenium on growth, survival, or percent of normal individuals (without deformities) of several aquatic species. The equations that described such functions were then used to estimate the concentration that promoted a 20 percent reduction in response observed at control levels (EC_{20}). These analyses were performed using the Toxic Effects Analysis Model software (version 0.02; R. Erickson, U.S. EPA Duluth).

Only data sets that met the following conditions were included in the analysis: (1) the experiment had a control treatment, which made it possible to define response levels at natural concentrations of selenium, (2) and at least four concentrations of selenium. (3) The highest tested concentration of selenium caused >50 percent reduction relative to the control treatment, and (4) at least one tested concentration of selenium caused <20 percent reduction relative to the control treatment to ensure that the EC_{20} was bracketed by tested concentrations of selenium. When the response was expressed as percentages (e.g., percent survival), transformed values (arcsin of the square root) were used to homogenize the variance.

Logistic regression assumes that a logistic model describes the log dose-response curve. For a visual display of such model, a logistic curve with three parameters was fitted to each data set using nonlinear least-squares regression analysis (Draper and Smith 1981). The logistic model was

$$y = \frac{y_0}{1 + ax^b}$$

where x symbolizes the selenium concentration in the organism's tissues, y is the response of interest (survival, growth, or reproduction), and y_0, a and b are model parameters estimated by the regression analysis. The y_0 parameter represents the response of interest at background levels of selenium. The graphs also include the 95 percent confidence interval for projections of the logistic model. These tasks were performed in S-Plus version 6.0 (Insightful 2001).

When the data from an acceptable chronic test met the conditions for of the logistic regression analysis, the EC_{20} was the preferred chronic value. When data did not meet the conditions, best scientific judgment was used to determine the chronic value. In this case the chronic value is the geometric mean of the NOAEC and LOAEC and termed the maximum allowable toxicant concentration (MATC). But when no treatment concentration was an NOAEC, the chronic value is less than the lowest tested concentration. And when no treatment concentration was a LOAEC, the chronic value is greater than the highest tested concentration.

Evaluation of Freshwater Chronic Data for Each Species

Acceptable freshwater chronic toxicity data are currently available for an aquatic invertebrate (*Brachionus calyciflorous*), eight different fish species, and a mix of fish species from the family Centrarchidae in a total of 21 distinct studies (Table 4). Detailed summaries of each study are included in Appendix I. Collectively, only these data were considered for the derivation of a final tissue residue criterion for selenium. Below is a brief synopsis of the experimental design, test duration, relevant test

endpoints, and other critical information regarding the derivation of each specific chronic value. The chronic toxicity values for other chronic selenium toxicity values and endpoints are included in Appendix I.

Brachionus calyciflorus (freshwater rotifer)

This study reported by Dobbs et al. (1996) is one of two laboratory-based experiments (also see Bennett et al. 1986) that involved exposing algae to selenium (in this case as sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fish (fathead minnows). In this particular study, the rotifers and fish were exposed to the same concentrations of sodium selenate in the water as the algae, but received additional selenium from their diet (i.e., the algae fed to rotifers and the rotifers fed to fish). The overall exposure lasted for 25 days. Rotifers did not grow well at concentrations exceeding 108.1 µg Se/L in water, and the population survived only 6 days at selenium concentrations equal to or greater than 202.4 µg Se/L in the water (40 µg Se/g dw in the algae). Regression analysis of untransformed growth data (dry weight) determined 4 day post-test initiation resulted in a calculated EC_{20} of 42.36 µg Se/g dw tissue (Table 4).

Oncorhynchus tshawytscha (chinook salmon)

Hamilton et al. (1990) conducted a 90-day growth and survival study with swim-up larvae fed one of two different diets. The first diet consisted of Oregon moist pellets where over half of the salmon meal was replaced with meal from selenium-laden mosquitofish (*Gambusia affinis*) collected from the San Luis Drain, CA (SLD diet). The second diet was prepared by replacing half the salmon meal in the Oregon moist pellets with meal from low-selenium mosquitofish (i.e., the same relatively uncontaminated mosquitofish that were used in the control diet) and spiked with seleno-DL-methionine (SeMe diet). Analysis of the trace element composition in the two different diets indicated that while selenium was the most toxic element in the SLD diet, concentrations of boron, chromium, iron and strontium in the high-selenium mosquitofish replacement diet (SLD diet type) were slightly elevated compared to the replacement diet composed of uncontaminated control mosquitofish that were spiked with organic selenium (SeMe diet type). These trace elements were, however, only 1.2 (e.g., iron) to 2.0 times (e.g., chromium) higher in the SLD diet than the SeMe diet, which contained the following measured concentrations (dry weight basis) in the food: boron- 10 µg/g; chromium- 2.8 µg/g, iron- 776 µg/g, and strontium- 48.9 µg/g.

During the test, the survival of control chinook salmon larvae and larvae fed the lowest dietary selenium concentrations in either dietary exposure type (SLD and SeMe, respectively, consuming food at approximately 3 µg Se/g dw) exceeded ≥97 percent up to 60 days post-test initiation. Between 60 and 90 days of exposure, however, the control survival declined significantly. Therefore, only data collected up to 60 days post-test initiation was considered for analysis. Regression analysis of untransformed growth data after 60 days of exposure resulted in a calculated EC_{20} of 15.74 µg Se/g dw tissue for fish fed the SLD diet type, and 10.47 µg Se/g dw tissue for fish fed the SeMe diet type (Table 4). Note: The mosquitofish from San Luis Drain were not tested for contaminants other than certain key elements suspected to be present in these fish. The San Luis Drain receives irrigation drainage from the greater San Joaquin Valley; and therefore, there is the possibility that the mosquitofish used in this study may have contained elevated levels of pesticides. The use of the SLD diet results assumes that selenium, and not these other possible contaminants, was the cause of any adverse chronic effects.

Oncorhynchus mykiss (rainbow trout)

Hilton and Hodson (1983) reared juvenile rainbow trout on either a high (25 percent) or low (11 percent) available carbohydrate diet supplemented with sodium selenite for 16 weeks. Body weights, feed:gain ratios, and total mortalities were followed throughout the exposure every 28 days. Tissues (livers and kidneys) were extracted for selenium analysis after 16 weeks. Fish fed the diets (low carbohydrate and high carbohydrate) with the highest selenium concentration (11.4 and 11.8 µg Se/g dw food, respectively) exhibited a 45 to 48 percent reduction in body weight (expressed as kg per 100 fish) compared to control fish by the end of the exposure, which the authors attributed to food avoidance. With only two dietary exposure concentrations and a control, these data were not amenable to regression analysis. The MATC for growth of juvenile rainbow trout relative to the final concentrations of selenium in liver tissue of trout reared on the high carbohydrate seleniferous dietary type is the geometric mean (GM) of 21.0 µg Se/g dw (NOAEC) and 71.7 µg Se/g dw (LOAEC), or 38.80 µg Se/g dw. Using the equation III to convert the selenium concentration in liver tissue to a concentration of selenium in the whole-body, the MATC becomes 11.65 µg Se/g dw (Table 4). The calculated MATC for the same group of experimental fish exposed to selenium in the low carbohydrate diet becomes 13.08 µg Se/g dw tissue, which is the same MATC for trout exposed for an additional 4 weeks based on the occurrence of nephrocalcinosis in kidneys (see Hicks et al. 1984; Appendix I).

Hilton et al. (1980) employed a similar test design as Hilton and Hodson (1983) in a later experiment to examine the narrow window at which selenium changes from an essential nutrient to a toxicant affecting

juvenile rainbow trout. The food consisted of a casein-torula yeast diet supplemented with selenium as sodium selenite. The experiment lasted for 20 weeks. During this time, the trout were fed to satiation 3 to 4 times per day, 6 days per week, with one feeding on the seventh day. Organs (liver and kidney) and carcasses were analyzed for selenium from fish sacrificed at 4 and 16 weeks. No gross histopathological or physiological effects were detected in the fish, although trout raised on the highest dietary level of selenium (13.06 µg Se/g dw) had a significantly lower body weight (wet basis), a higher feed:gain ratio, and higher number of mortalities (10.7; expressed as number per 10,000 fish days). The MATC for growth and survival of juvenile rainbow trout relative to the final concentrations of selenium in liver tissue is the GM of the NOAEC (40 µg Se/g dw tissue) and the LOAEC (100 µg Se/g dw tissue), or 63.25 µg Se/g dw. Using equation III to convert selenium concentrations in the liver to selenium concentrations in the whole body, the MATC becomes 19.16 µg/g dw (Table 4).

Eggs and milt were obtained from ripe rainbow trout collected from reference streams and streams containing elevated selenium from an active coal mine in Alberta, Canada (Holm 2002; Holm et al. 2003). Eggs were fertilized and monitored in the laboratory until swim-up stage for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. Similar investigations were conducted in 2000 and in 2001. The effort in 2001 added a stream with an intermediate level of selenium contamination and another reference stream. The only other notable difference between 2000 and 2001 was the temperature at which the embryos were incubated; 8°C in 2000 and 5°C in 2001. The author stated 5°C more closely approximated actual incubation temperatures for rainbow trout eggs. No differences were observed for percent fertilization or mortality between the reference and contaminated sites in both the 2000 and 2001 investigations. The frequencies of embryonic deformities and edema were significantly greater in the stream affected by coal mining than in the reference stream in the 2000 study. The average frequencies of embryonic craniofacial, skeletal and finfold deformities in the contaminated stream were 7.7, 13.8 and 3.2 percent, respectively; the average frequency of edematous embryos was 30.8 percent. The effect level for selenium was determined to be the average selenium concentration in rainbow trout muscle tissue, 1.50 µg Se/g ww. Muscle ww was converted to dw using 75.84 percent moisture derived for rainbow trout and equation 1 was used to convert selenium muscle dw to selenium in whole body dw. The chronic value determined for embryonic abnormalities in rainbow trout (2000 study) was 5.79 µg Se/g adult whole body dw. A comparison of the frequency of embryonic deformities or edema between selenium contaminated and reference streams with the 2001 data indicated there were no significant differences. An EC_{20} value, however, was computed for the relationship between craniofacial deformities and the concentration of

selenium in eggs, 10.4 µg Se/g eggs ww. Quantile regression was used to convert selenium in egg ww to muscle ww using the rainbow data reported by Holm et al. (2003). The remaining conversion to the whole body dw value of 5.85 µg Se/g was made using 75.84 percent moisture and equation 1. See Appendix I for details on these studies.

Oncorhynchus clarki (cutthroat trout)

No significant effects of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry from wild-caught cutthroat trout from a reference and exposed site (Fording River, British Columbia, Canada) were observed by Kennedy et al. (2000). The observations were made on eggs reared in well water from spawning age females collected from the two locations (N = 17 and 20, respectively) and fertilized by one male collected at each site. The mean selenium content in muscle tissue from adult fish was 2.4 µg/g dw tissue for fish collected from the reference site, and 12.5 µg/g dw tissue for fish collected from the Fording River. Using Equation I to convert the selenium concentration in muscle tissue to a selenium concentration in the whole-body, the chronic value for this study was estimated to be >10.92 µg/g dw parental fish tissue (see Table 4).

Hardy (2002) fed cutthroat trout experimental diets containing a range of selenomethionine (0-10 µg/g dw) for 124 weeks. No significant growth or survival effects were observed in the adult fish over the 124 weeks which reached a whole body concentration of 12.5 µg/g dw selenium after 44 weeks. Embryo-larval observations (percent hatch and percent deformed) were not related to whole body selenium concentrations in the spawning females (9.37 µg/g dw) fed the selenium-laden diet for 124 weeks. The chronic value for this study was determined to be >9.37 µg Se/g dw.

Salvelinus fontinalis (brook trout)

Spawning brook trout were collected from streams with elevated selenium contaminated by coal mining activity and from reference streams in 2000 and again in 2001 (Holm 2002; Holm et al. 2003) . Similar to that described for rainbow trout above, fertilized eggs were monitored in the laboratory for percent fertilization, deformities (craniofacial, finfold, and spinal malformations), edema, and mortality. The only abnormality observed in the embryos spawned from the brook trout collected in 2000 at the contaminated stream that had a frequency greater than the reference stream was craniofacial deformity (13.6 percent for the contaminated stream compared to 3.0 percent in the reference stream). The effect level for craniofacial deformity in brook trout for the 2000 data was determined to be the average selenium concentration in adult muscle tissue, 3.79 µg Se/g ww or 13.2 µg Se/g whole body dw using

conversion factors (75.84 percent moisture and equation 1). The only significant difference observed in 2001 brook trout was a greater frequency of finfold deformities in brook trout collected from Gregg Creek (intermediate selenium levels) relative to the reference stream (4.1 percent in Gregg Creek compared to 0.1 percent in the reference stream). The effect level for finfold deformites in the 2001 study was estimated to be the concentration of selenium in brook trout eggs from Gregg Creek, 6.88 µg Se/g ww. Using the same conversion factors used for rainbow trout in the Holm study described above, the chronic value in adult whole body dw is 12.4 µg Se/g. See Appendix I for more details.

Salmonidae summary

Four of the studies with salmonids discussed above evaluated the effects of selenium directly on growth of juvenile fish (Hamilton et al. 1990; Hilton and Hodson 1983; Hilton et al. 1980; Hicks et al. 1984), while three of the studies evaluated the effects of selenium on embryo/larval survival and deformity where exposure was through the parents (Hardy et al. 2002; Holm 2000; Holm et al. 2003; Kennedy et al. 2000). Of the studies based on embryo/larval survival and deformity where exposure was through the parents, fry from hatchery brood fish were fed a selenium-spiked diet, grown to sexual maturity, and spawned for the effects determination in the Hardy et al. study, and wild-caught adults from selenium contaminated streams were spawned for the effects determination in the Holm studies and in the Kennedy et al. study. Significant effects due to selenium exposure in these field exposed studies were not observed for cutthroat trout (Hardy et al. 2002; Kennedy et al. 2000). Significant effects were observed for rainbow trout and brook trout, albeit relatively minor effects in the latter species (Holm 2002; Holm et al. 2003). Although significant effects were not observed in the Hardy et al. and Kennedy et al. studies, the data are meaningful with respect to the effect levels obtained for embryo-larval development in *Oncorhynchus*, and thus retained for GMCV (10.66 µg Se/g dw) calculation (Table 4).

Pimephales promelas (fathead minnows)

Chronic values for fathead minnows were derived from three laboratory-based studies and one mesocosm study (Table 4). Two of the laboratory studies (Bennett et al. 1986 and Dobbs et al. 1996) involved exposing algae to selenium (either as sodium selenite or sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fathead minnows. In the Bennett et al. (1986) study, larval fathead minnows were fed control (cultured in chambers without selenium containing algae) or selenium-contaminated rotifers (cultured in chambers with selenium containing algae previously exposed to sodium selenite in the water) in three separate experiments lasting 9 to 30 days. The different experiments were distinguished by: 1) the day selenium-laden rotifers were first fed, 2) the day selenium-

laden rotifers were last fed, and 3) the age of larvae at experiment termination. The results from the three experiments reported by Bennett et al. (1986) were conflicting. Larval growth was significantly reduced at whole-body selenium concentrations ranging from 43.0 to 51.7 µg/g dw tissue in the first two experiments (see Appendix I for conditions), but growth was not significantly reduced in larvae that had accumulated 61.1 µg/g dw tissue in the third experiment (Table 4). The geometric mean of these three values, 51.40 µg/g dw, was considered the chronic value for selenium for this test.

A similar test system was used by Dobbs et al. (1996), in which larval fathead minnows were exposed to the same concentrations of sodium selenate in the water as their prey (rotifers), but also received additional selenium from the consumption of the selenium-contaminated rotifers. In this study, the fathead minnows did not grow well at concentrations exceeding 108.1 µg Se/L in water, and they survived only to 11 days at selenium concentrations equal to or greater than 393.0 µg/L in the water (75 µg Se/g dw in the diet, i.e., rotifers). The LOAEC for retarded growth (larval fish dry weight) in this study was <73 µg Se/g dw tissue (Table 4).

In contrast to the above laboratory-based food chain studies, Ogle and Knight (1989) examined the chronic effects of only elevated foodborne selenium on growth and reproduction of fathead minnows. Juvenile fathead minnows were fed a purified diet mix spiked with inorganic and organic selenium in the following percentages: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine. The pre-spawning exposure lasted 105 days using progeny of adult fathead minnows originally obtained from the Columbia National Fishery Research Laboratory, and those obtained from a commercial fish supplier. After the 105 day exposure period, a single male and female pair from each of the respective treatment replicates were isolated and inspected for spawning activity for 30 days following the first spawning event of that pair. There was no effect from selenium on any of the reproductive parameters measured, including larval survival, at the dietary concentrations tested (5.2 to 29.5 µg Se/g dw food). Sub-samples of larvae from each brood were maintained for 14 days post-hatch and exhibited >87.4 percent survival. The pre-spawning adult fish fed a mean dietary level of 20.3 µg Se/g dw did exhibit a significant reduction in growth compared to controls (16 percent reduction), whereas no effect on growth occurred in the fish fed 15.2 µg Se/g dw. The whole-body chronic value, as determined by the GM of the NOAEC and the LOAEC measured at 98 days post-test initiation, was 5.961 µg/g dw tissue (Table 4).

The chronic value of 5.961 µg/g dw determined for growth after 98 days of exposure to pre-spawning fathead minnow adults (Ogle and Knight, 1989) was approximately an order of magnitude lower than the

growth effects to fathead minnow observed in Bennett et al. (1986) and Dobbs et al (1996). The length of exposure in the Ogle and Knight test was more than twice as long as either Bennett et al. or Dobbs et al., suggesting a longer duration was needed in order to detect any growth effects from selenium. However, in addition to the absence of effects observed for the reproductive parameters measured, survival of larvae hatched from parents exposed to each of the five selenium treatments (including those in which growth was affected) was not affected.

Other studies (Bryson et al. 1984; Bryson et al. 1985a; Coyle et al. 1993; Hermanutz et al. 1996) have found larval deformities and larval survival to be the most sensitive endpoint to fish. This also appears true for fathead minnows. Schultz and Hermanutz (1990) examined the effects of selenium in fathead minnow larvae transferred from parental fish (females). The parental fathead minnows were originally exposed to selenite which was added to artificial streams in a mesocosm study. The selenite entered the food web which contributed to exposure from the diet. Spawning platforms were submerged into treated and control streams. The embryo samples that were collected from the streams were brought into the laboratory and reared in incubation cups which received stream water dosed with sodium selenite via a proportional diluter. Edema and lordosis were observed in approximately 25 percent of the larvae spawned and reared in natural water containing 10 µg Se/L. Selenium residues in the ovaries of females from the treated stream averaged 39.27 µg/g dw. Using equation II to convert the selenium concentration in the ovaries to a concentration of selenium in the whole-body, the chronic value for this species was estimated to be <18.21 µg Se/g dw (Table 4).

Since Ogle and Knight reported that food in the higher selenium concentrations remained uneaten and fish were observed to reject the food containing the higher selenium concentrations, the authors suggested that the decreased growth was caused by a reduced palatability of the seleniferous food items. This is a common observation also noted by Hilton and Hodson (1983) and Hilton et al. (1980) and apparent in Coughlan and Velte (1989). Given the no observed effect to larval survival and the apparent non-toxicological effect on growth in the Ogle and Knight study, the SMCV for fathead minnows does not include the 5.961 µg/g dw chronic value.

Also excluded from the SMCV calculation for fathead minnows were the chronic value and LOAEC estimated from the laboratory food-chain experiments of Bennett et al. (1986) and Dobbs et al. (1996). In both of these studies, the effect concentrations based on larval growth appear to be less sensitive than the effect on larval edema and deformity observed in Schultz and Hermanutz (1990). The greater

sensitivity of larval fathead minnows to selenium as measured by edema and deformity (lordosis) in the Schultz and Hermanutz (1990) study is consistent with other studies using bluegill (Table 4); and thus, the SMCV for fathead minnows of <18.21 µg/g dw was based on this endpoint.

Catostomus latipinnis (flannelmouth sucker)

Beyers and Sodergren (2001a) exposed flannelmouth sucker larvae to a range of aqueous selenate concentrations (<1, 25.4, 50.6, 98.9, and 190.6 µg/L) and respectively fed them a range of selenium in their diet (rotifers containing <0.702, 1.35, 2.02, 4.63, and 8.24 µg/g dw). There were no survival or growth effects observed after the 28 day exposure. The chronic value based on the concentration of selenium measured in the larvae exposed to the highest test concentration was >10.2 µg Se/g dw.

Xyrauchen texanus (razorback sucker)

Two laboratory exposure studies have been done with the endangered razorback sucker. In the first study, Beyers and Sodergren (2001a) exposed larval razorback suckers to the same aqueous and diet concentrations as described above for the flannelmouth sucker. Similar to the results found for the flannelmouth sucker, survival and growth of the razorback sucker larvae were not reduced after the 28 day exposure. The chronic value for this study based on selenium measured in the larvae at the end of the test is > 12.9 µg Se/g dw. In a second study, Beyers and Sodergren (2001b) exposed larval razorback suckers to a control water and three different site waters containing varying concentrations of selenium. Two treatments were tested within each water type, fish fed rotifers cultured in the same water type (site diet) and fish fed rotifers cultured in control water. There were no reductions in survival or growth in fish exposed to both the site water and site diet compared to fish exposed to control water and control diet. There were, however, reductions in growth in fish exposed to site water/site food compared to the same site water and control food. The authors did not attribute the effect on larval growth by the diet to selenium and cited several lines of evidence, including: (1) there was not a dose-response relationship in the concentration of selenium in the food (rotifers) and growth, nor in the concentration of selenium in the fish larvae and growth across the three water types; and (2) the site water type, identified as De Beque, showed a significant reduction in the growth of fish exposed to site water/site food relative to site water/control food, but contained levels of selenium in the water (< 1µg/L) and food (2.10 µg/g dw) typically lower than those that have been found to elicit effects. The chronic value for this study is > 42 µg Se/g dw based on the whole body concentration of selenium in the larval razorback suckers exposed to North Pond site water.

Lepomis macrochirus (bluegill sunfish)

Applicable chronic data for bluegill sunfish can be grouped according to field exposure versus laboratory exposure. In some field studies, chronic tolerance to selenium appears to be much higher than in laboratory studies (Bryson et al. 1985a). In the Bryson et al. (1984, 1985a) and Gillespie and Baumann (1986) studies, the progeny of females collected from a selenium contaminated reservoir, Hyco Reservoir, Person County, NC and artificially crossed did not survive to swim-up stage, irrespective of the origin of milt used for fertilization. Measured waterborne selenium concentrations prior to the experiments ranged from 35 to 80 µg/L. The whole-body tissue selenium concentration in the female parent associated with this high occurrence of mortality of hatched larvae was <43.70 µg/g dw tissue, as reported by Bryson et al. (1985a), and <21.47 µg/g dw tissue, as reported by Gillespie and Baumann (1986) (Table 4). In the case of the latter, nearly all swim-up larvae from the Hyco Reservoir females were edematous, none of which survived to swim-up. These chronic effect tissue values are in line with the EC_{20} calculated for the occurrence of deformities among juvenile and adult fishes from the family Centrarchidae collected from Belews Lake, NC, i.e., 44.57 µg Se/g dw (see Lemly 1993b, Table 4).

Bryson et al. (1985b) conducted juvenile survival toxicity tests using hatchery bluegill and various forms of selenium spiked to an artificial diet as well as a diet consisting of zooplankton collected from Hyco Reservoir. There was no effect on length or weight of the juvenile bluegill after 60 days of exposure. The highest concentration of selenium measured in whole body fish tissues in these tests was in the seleno-DL-cysteine-2X treatment (3.74 µg Se/g dw). Bryson et al. (1985b) also examined percent hatch and percent swim-up larvae from spawns using fish collected from Hyco Reservoir and a control site. There were no differences in the Hyco measurements relative to the control. The concentration of selenium in the liver of the parental Hyco bluegill was 18.6 µg/g dw or 5.45 µg Se/g dw whole body using equation III for conversion. The chronic values for the juvenile bluegill test and the embryo-larval development tests were >3.74 and >5.45 µg Se/g dw whole body, respectively.

In contrast, the chronic effects threshold for larval survival in a combination laboratory waterborne and dietary selenium exposure (Coyle et al. 1993), or even a long-term mesocosm exposure (Hermanutz et al. 1996), occurs at concentrations approximately 3 times lower than those recorded above (Table 4). In the Coyle et al. (1993) study, two-year old pond reared bluegill sunfish were exposed in the laboratory to a nominal 10 µg Se/L in water (measured concentrations in respective dietary treatments ranging from 8.4 to 11 µg/L) and fed (twice daily *ad libitum*) Oregon moist pellets containing increasing concentrations of seleno-L-methionine. The fish were grown under these test conditions for 140 days. Spawning

frequency, fecundity, and percentage hatch were monitored after 60 days when spawning began to occur. There was no effect of the combination of the highest dietary selenium concentration (33.3 µg Se/g dw) in conjunction with waterborne selenium concentrations averaging 11 µg/L on adult growth, condition factor, gonadal somatic index, or the various reproductive endpoints (Appendix I). The survival of newly hatched larvae, however, was markedly reduced; only about 7 percent survived to 5 days post-hatch. Regression analysis on arcsin square root transformed fry survival data 5 days post-hatch resulted in a calculated EC_{20} of 8.954 µg Se/g dw tissue (Table 4).

Hermanutz et al. (1996), as corrected by Tao et al. (1999), and peer reviewed in Versar (2000), exposed bluegill sunfish to sodium selenite spiked into artificial streams (nominal test concentrations: 0, 2.5, 10, and 30 µg Se/L) which entered the food web, thus providing a simulated field-type exposure (waterborne and dietary selenium exposure). A series of three studies were conducted over a 3 year period lasting anywhere from 8 to 11 months. All three studies began exposure to adult bluegill sunfish in the fall and ended the respective study in the summer of the following year. Winter temperatures averaged 4.1 and 4.5°C and spawning months (June-July) averaged 23.9 and 22.4°C, respectively for Studies II and III. The Hermanutz et al. (1996) report contains the data presented in the Hermanutz et al. (1992) article (Study I, 10 and 30 µg/L exposures) as well as Studies II and III (2.5 and 10 µg/L and recovering mesocosms). Spawning activity was monitored in the stream, and embryo and larval observations were made *in situ* and from fertilized eggs taken from the streams and incubated in egg cups in the laboratory. None of the adult bluegill exposed to the highest concentration of selenium in the water (Study I, mean measured concentration equal to 29.4 µg/L) survived. Incidence of edema, hemorrhage, and lordosis in the larvae incubated in egg cups and spawned from fish exposed to 10 µg Se/L were 100, 45 and 15 percent, respectively (see Hermanutz 1996 in Appendix I). Such health problems were not observed in larvae from fish that were not exposed to elevated concentrations of selenium (control treatment). Rates of edema, hemorrhage, and lordosis occurrence in larvae (egg cup data) from fish exposed to 2.5 µg Se/L were 0, 3.6 and 0 percent, respectively. Mean concentrations of selenium in fish tissues (whole body) of the control, 2.5 and 10 µg Se/L treatments were 1.95, 5.55, and 26.46 µg Se/g dw, respectively. Except for the 2.5 µg Se/L treatment, each value is a geometric mean of 2 replicates.

Results of this experiment were not suitable for regression analysis. Exposure of adult fish to 10 µg Se/L caused a small reduction in larval survival (in their first three days), from 75 to 57 percent. However, responses lower than half of the values observed in control treatments are needed to adequately characterize the slope of decline in survival (or growth, reproduction...) with increasing concentrations of

selenium. It is not sufficient to have only extremely low and high responses. Intermediate effects are necessary to properly estimate the shape of the dose-response curve. The percent of larvae with edema increased from 0 percent at the control and 2.5 µg Se/L treatments to 100 percent in streams that received 10 µg Se/L. With these data, it is not possible to accurately estimate the lowest concentration with adverse effects (LOAEC) nor the rate at which incidence of edema increases with higher tissue concentrations of selenium.

The chronic value for this study was estimated from results of analysis of variance (ANOVA) reported by Tao et al. (1999). ANOVA was utilized to evaluate effects of elevated concentrations of selenium on percent hatch, percent survival, maximum percent edema, lordosis, and hemorrhage, and minimum percent healthy (egg cup data). Treatment effects were only significant for maximum percent edema and minimum percent healthy (see their Table 4-19), and in no instance were differences between the 2.5 µg Se/L and control treatments significant (Dunnett's Means test, all probabilities > 0.1, see their Table 4-20). These results clearly suggest that the 2.5 µg Se/L treatment had no adverse impact on bluegill larvae. They are further supported by analysis of the field nest data (see Hermanutz 1996 in Appendix I). In this experiment, treatment had a significant effect on maximum percent edema (raw data and ranks) and maximum percent hemorrhage (ranks only). Probabilities of differences between the 2.5 µg Se/L and control treatments were >0.2 for all response variables except maximum percent hemorrhage, which had an estimated probability of 0.05 (raw data, P=0.022 for ranks; Dunnett's means test). Such values, though, were well above the adjusted experiment-wise error rate for multiple comparisons (α'=0.0085, $\alpha'=1-(1-\alpha)^{1/k}$; α=0.05, k=6 comparisons; Sokal and Rohlf 1981), which takes into account the fact that selenium effects were tested on six different response variables. Therefore, the chronic value for this study, 12.12 µg Se/g dry weight, was calculated as the geometric mean of tissue concentrations of selenium in the 2.5 (NOAEC) and 10 µg Se/L (LOAEC) treatments (5.55 and 26.46 µg/g dw, respectively).

The importance of diet in the bioaccumulation of selenium was demonstrated in one additional experiment. Study III consisted of the addition of new adult bluegill to the same streams that received the 2.5, 10 and 30 µg/L sodium selenite during previous studies, but with all dosing of selenite halted. The adult bluegills exposed only to dietary selenium present in the food web accumulated selenium to levels very near to the levels accumulated during Study II in which aqueous selenium was also present demonstrating the importance of diet on selenium accumulation. There were no effects (no effect on larval survival, 0 percent deformities, 0 percent hemorrhaging), on the bluegill progeny in Study III even

from fish that accumulated 11.7 and 14.5 µg/g dw in the recovering 10 µg/L streams, and 17.35 µg/g dw in the recovering 30 µg/L stream. The lack of any effect on the Study III larvae suggests that although dietary exposure would have been the predominant exposure route in both Study II and Study III, environmental differences influenced the toxicological significance of the tissue concentrations.

A 90-day diet-only laboratory exposure in which juvenile bluegill sunfish were fed a range of selenomethionine concentrations added to Oregon moist did not have any significant effects on survival (Cleveland et al. 1993). The authors did report a significant decrease in the condition factor (K) at the diet treatment where bluegill whole body tissue concentrations were measured at 7.7 µg Se/g dw. The condition factor (weight x 10^5/length^3) is reflective of the weight of the fish, and as discussed earlier, the avoidance of food at similar dietary concentrations in other fish studies (Ogle and Knight 1989; Hilton and Hodson 1983; Hilton et al. 1989; Coughlan and Velte 1989) suggests the reduction in K is possibly a non-toxicological effect. Given the very slight reduction in K (1.3 to 1.2) and the uncertain relevance of growth data, the chronic value for this study was estimated at > 13.4 µg Se/g dw.

Data from Lemly (1993a) indicate that over-wintering fish may be more susceptible to the effects of waterborne and dietary selenium due to increased sensitivity at low temperature. The authors exposed juvenile bluegill sunfish in the laboratory to waterborne (1:1 selenite:selenate; nominal 5 µg Se/L) and foodborne (seleno-L-methionine in TetraMin; nominal 5 µg Se/g dw food) selenium for 180 days. Tests with a control and treated fish were run at 4^{O}C and 20^{O}C with biological and selenium measurements made every 60 days. Survival and whole-body lipid content were unaffected at 20^{O}C (whole-body selenium concentrations equal to 6 µg/g dw) when compared to control fish. Fish exposed to the combination low-level waterborne and dietary selenium at 4^{O}C exhibited significantly elevated mortality (40.4 percent) relative to controls (2.9 percent), and exhibited significantly greater oxygen consumption and reduced lipid content, which are all indicative of an additional stress load. The chronic value for juvenile bluegill sunfish exposed to waterborne and dietary selenium at 4^{O}C was <7.9 µg/g dw tissue, whereas the chronic value for juvenile bluegill sunfish exposed to waterborne and dietary selenium based on survival at 20^{O}C was >6 µg/g dw whole-body tissue.

Five of the studies discussed above evaluated the effects of selenium on fish larvae to which exposure was through the parents. Three of these studies collected adult fish from Hyco Reservoir to which the bluegill population had been exposed to elevated selenium concentrations for multiple generations (Bryson et al. 1984; Bryson et al. 1985a; Gillespie and Baumann 1986), whereas the other two studies

exposed bluegill parents obtained from an uncontaminated source (Coyle et al. 1993; Hermanutz et al. 1996). The average of the chronic values reported for the Hyco studies was four times greater than the value in the latter two studies. This difference may simply be the inability of the field tests to detect a lower effect concentration than that which was observed at the site. However, Bryson et al. (1985a) found no effects to larval survival from Hyco Reservoir females collected in an "unaffected area" containing 19.18 µg Se/g dw suggesting the possibility of tolerance through physiological or genetic adaptation of the previous exposed bluegill population at Hyco Reservoir.

Acquisition of tolerance to selenium has also been implied in the literature for other fish species. For example, Kennedy et al. (2000) suggested tolerance at the cellular level as an explanation for the normal development of early life stages for cutthroat trout collected from a stream containing 13.3 to 14.5 µg Se/L in the water column. These authors reported that the overall frequency of larval deformities in the exposed population was less than 1 percent, and in one fish containing eggs with 81.3 µg Se/g dw, there were 0.04 percent pre-ponding deformities and 3.3 percent larval mortalities. It should be noted that the acquisition of tolerance to selenium has been hypothesized (Kennedy et al. 2000), but has not yet been substantiated. Other than the Kennedy et al. study, tolerance to selenium in one of the endpoints consistently sensitive to fish (embryo-larval development) has not been reported in the literature and its reality is uncertain at this time. However, given the need to protect sensitive populations of species, the chronic values for the studies in which eggs and larvae were obtained from bluegill adults that were exposed to elevated selenium for multiple generations (i.e., Bryson et al. 1984; Bryson et al. 1985a; Gillespie and Baumann, 1986) were not included in the SMCV calculation.

Morone saxitilis (Striped bass)

The only remaining applicable chronic value for selenium was determined from a laboratory dietary exposure conducted using yearling striped bass (Coughlan and Velte 1989). During the experiment, the bass were fed contaminated red shiners (38.6 µg Se/g dw tissue) from Belews Lake, NC (treated fish) or golden shiners with low levels of selenium (1.3 µg/g dw tissue) purchased from a commercial supplier (control fish). The test was conducted in soft well water and lasted up to 80 days. During the experiment, all fish were fed to satiation 3 times per day. Control fish grew well and behaved normally. Treated fish behaved lethargically, grew poorly due to a significant reduction in appetite, and showed histological damage, all eventually leading to the death of the animal. The final selenium concentration in muscle of treated striped bass averaged from 17.50 to 20.00 µg/g dw tissue (assuming 80 percent moisture content), which was 3.2 to 3.6 times higher than the final selenium concentrations in control

striped bass, which averaged 5.500 µg/g dw tissue. Using equation I to convert the selenium concentration in muscle tissue to a selenium concentration in the whole-body, the chronic value for this species was determined to be <14.75 µg/g dw (Table 4).

Formulation of the Final Chronic Value (FCV) for Selenium

The lowest GMCV in Table 4 is for bluegill, 9.500 µg/g dw whole body, which is the geometric mean of chronic values from the laboratory study of Coyle et al. (1993), the laboratory study of Lemly (1993a) and the macrocosm exposure study of Hermanutz et al. (1996). Several of the chronic values listed in Table 4 were not used in the calculation of this GMCV. These values fall under several categories. The "less than" values tabulated for Bryson et al. (1984) and Gillespie and Baumann (1986) for Hyco Reservoir bluegill were not used to because they only indicate a chronic value in a range that includes 9.500 µg/g dw. The "greater than" values for Bryson et al (1985b) were not used because similar studies with bluegill sunfish provided more meaningful information on effect levels. The "greater than" value for the recovering systems in Study III from Hermanutz et al. (1996) was not used in the mean calculation because, as previously discussed in the *Lepomis* section, less tolerance was observed in the freshly exposed systems of Study II. The Table 4 results for Bryson et al. (1985a) and Lemly (1993b) were also not used in calculating the bluegill GMCV. Bryson et al. (1985a) indicated a chronic value for Hyco Reservoir bluegill somewhere between 20.29 and 43.70 µg/g dw. Lemly (1993b), appearing in Table 4 under the category Centrarchidae, the family to which bluegill belong, yielded a chronic EC_{20} of 44.57 µg/g dw specific for fish from Belews Lake, NC, again substantially above the GMCV of 9.500 µg/g dw. It is not known whether historical exposure to elevated selenium concentrations, such as occurred at Belews Lake and Hyco Reservoir, will dependably lead to this magnitude of increase in the chronic tolerance of resident fish.

The Lemly (1993a) laboratory results, indicating a chronic value for over-wintering juvenile bluegill sunfish of <7.91 µg/g dw, are not completely comparable to the other values used to calculate the bluegill GMCV. This study involved an additional natural stress, exposure to a simulated winter low temperature of 4°C. In this study, juvenile bluegill sunfish exposed to the over-wintering temperature 4°C appeared to accumulate more selenium in whole-body tissues (7-8 µg Se/g dw tissue) relative to those exposed at 20°C (5-6 µg Se/g dw tissue), but also exhibited increased signs of chronic toxicity. Because this stress occurs annually to one degree or another in nearly all the country, the FCV was lowered to 7.91 µg/g dw to protect sensitive fish species Although the literature contains little information on the temperature-dependence of selenium toxicity, Lemly's study (further summarized in Appendix I) was judged to be

sufficiently definitive to merit lowering the FCV. The study showed a clear effect on juvenile bluegill survivorship when tissue concentrations reach 7.91 µg Se/g dw under extended cold temperature conditions.

In the Lemly (1993a) study, the author relates the selenium induced hematological changes to gill lamellar damage (possible reasons cited were the collection of cell parts in capillaries restricting blood flow increasing pressure and rupturing or swelling lamellar vessels, and smaller red cells becoming tightly packed in vessels). The author postulates that an imbalance between respiratory demands (i.e., Se-exposed fish used more O_2 at both 4°C and 20°C) and decreased respiratory capacity could have constituted a stress that resulted in reduced body condition and lipid content of fish in the cold treatment. The condition of the combination of selenium-induced elevation in energy demand and reductions in feeding due to cold temperature and short photoperiod, leading to severe depletion of stored body lipid was termed, Winter Stress Syndrome.

The Guidelines indicate that the chronic criterion (in this case the FCV) is intended to be a good estimate of the threshold for unacceptable effect. The Guidelines point out that the threshold for unacceptable effect does not equate with a threshold for any adverse effect. For example, some adverse effects, possibly even a small reduction in survival, growth, or reproduction may occur at this threshold. If over-wintering bluegill are as sensitive as indicated by the Lemly (1993a) results, some reduction in survival (compared to populations accumulating lesser concentrations of selenium or exposed to less severe winter temperatures) would occur at the FCV. Nevertheless, other studies, those of Lemly (1993b) and Bryson et al. (1985a), suggest that historically exposed populations may not be as sensitive as the organisms studied by Lemly (1993a).

The bluegill exposed to selenium at 4°C in the Lemly (1993a) study accumulated 7.91 µg/g dw, whereas those exposed to Se at 20°C accumulated only 5.74 µg/g dw. The increase in the concentration of selenium in whole body tissue at 4°C was apparently due to reductions in lipid and body weight caused by decreased feeding by the juvenile bluegill resulting in a concentration of selenium in their tissues. If this concentration of selenium in tissues occurs in sensitive overwintering fish in nature, a criterion of 5.85 µg/g dw (the selenium tissue concentration in the 4°C exposure after 60 days) in for fish collected during the summer or fall months might be warranted to protect the selenium-sensitive fish during the winter months. However, it is not understood at this time whether fish in nature do concentrate selenium during the winter. The Lemly (1993a) study used an artificial diet spiked with seleno-methionine.

Although the 20°C fish did not show signs of food avoidance to the Se-spiked food, as discussed earlier in this section, other studies did observe decreased feeding and effects on growth.

If sensitive juvenile fish are indeed adversely affected during winter months, field studies should indicate an altered age structure relative to selenium whole body tissue levels. May et al. (2001) reported that an analysis of the size structure of bluegill populations in the Republican River and in 7 reservoirs within this river's basin, where mean tissue concentrations ranged from 2.85 to 8.84 mg Se/g dw, revealed large numbers of small fishes. Similar patterns in the size structure of fish populations were observed for 7 additional species: common carp, green sunfish, channel catfish, largemouth bass, gizzard shad, black bullhead, and river carpsucker.

Given the uncertainty of juvenile fish concentrating selenium over the winter, an FCV of 7.91 µg Se/g dw is recommended. However, if the concentration of selenium in whole body fish tissues approaches 5.85 µg Se/g dw during the summer or fall months, it is recommended fish be sampled during the winter to determine if they exceed the FCV of 7.91 µg Se/g dw.

The FCV may not necessarily protect fish in a hypothetical environment where they are exposed only via water and not via diet. If the organisms are provided with an uncontaminated diet, then exceedingly high water concentrations, possibly above the acute criterion, are needed to elicit effects, but such effects may occur at tissue concentrations below the FCV (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978). This is not a practical limitation, however, since water-only exposure of selenium is not representative of the actual exposure of selenium to aquatic organisms in the environment.

The FCV of 7.91 µg/g dw was based on a scientific interpretation of the data presented in Table 4. Although the FCV is derived from a limited number of species (9 species/7 genera), it is intended to be protective of aquatic organisms across the United States. There may be aquatic communities whose fish assemblage may contain species with different sensitivities to selenium compared to those listed in Table 4. Furthermore, even within the Table 4 bluegill data, there is a range of reported tissue NOAECs from various sites. Consequently, results from appropriate site-specific studies could be used to modify the criterion.

A comparison of the FCV to tissue values measured in U.S. Fish and Wildlife Service's National Contaminant Biomonitoring Program and U.S. Geological Survey's National Water Quality Assessment (NAWQA) program is provided in Appendix J.

Table 4. Freshwater Chronic Values from Acceptable Tests

Species	Reference	Exposure route	Selenium form	Toxicological endpoint	Chronic value, µg/g dw[a]	SMCV µg/g dw	GMCV µg/g dw
Brachionus calyciflorus rotifer	Dobbs et al. 1996	dietary and waterborne (lab)	algae exposed to SeVI in water, algae then fed to rotifers	EC_{20} for rotifer dry weight after 4 d	42.36	42.36	42.36
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab)	Se-laden mosquitofish from San Luis Drain, CA	EC_{20} for juvenile growth	15.74 (juvenile tissue)	12.84	10.66
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab)	Mosquitofish spiked with seleno-DL-methionine	EC_{20} for juvenile growth	10.47 (juvenile tissue)		
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983: Hicks et al. 1984	dietary (lab)	sodium selenite in food preparation	MATC for juvenile growth; nephrocalcinosis	11.65[b] (juvenile tissue)	9.32	
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab)	sodium selenite in food preparation	MATC for juvenile survival and growth	19.16[b] (juvenile tissue)		
Oncorhynchus mykiss rainbow trout	Holm 2000; Holm et al. 2003	dietary and waterborne (field Luscar River, Alberta)	not determined	2000 study: chronic value for embryo larval deformities 2001 study: EC_{20} for craniofacial deformities	5.79[c] (parent tissue) 5.85[c] (parent tissue)		
Oncorhynchus clarki cutthroat trout	Kennedy et al. 2000	dietary and waterborne (field - Fording River, BC)	not determined	NOAEC for embryo/larval deformities and mortality	>10.92[c] (parent tissue)	>10.12	
Oncorhynchus clarki cutthroat trout	Hardy, R.W. 2002	dietary (lab)	selenomethionine in food preparation	NOAEC for embryo/larval deformities	>9.37 (parent tissue)		

Species	Reference	Exposure route	Selenium form	Toxicological endpoint	Chronic value, µg/g dw[a]	SMCV µg/g dw	GMCV µg/g dw
Salvelinus fontinalis brook trout	Holm 2002; Holm et al. 2003	dietary and waterborne (field Luscar River, Alberta)	not determined	2000 study: chronic value for craniofacial deformities 2001 study: chronic value for finfold deformities	13.2[c] (parent tissue) 12.4[c] (parent tissue)	12.8	12.8
Pimephales promelas fathead minnow	Bennett et al. 1986	dietary (lab)	algae exposed to selenite then fed to rotifers which were fed to fish	Chronic value for larval growth	51.40[d] (larval tissue)	<18.21	<18.21
Pimephales promelas fathead minnow	Ogle and Knight 1989	dietary (lab)	mix of 25, 50, and 25 percent selenate, selenite, and seleno-L-methionine in food preparation	MATC for pre-spawning adult growth	5.961[d] (pre-spawning adult tissue)		
Pimephales promelas fathead minnow	Dobbs et al. 1996	dietary and waterborne (lab)	algae exposed to selenate in water then fed to rotifers which were fed to fish	LOAEC for larval fish dry weight after 8 d	<73[d] (larval tissue)		
Pimephales promelas fathead minnow	Schultz and Hermanutz 1990	dietary and waterborne (mesocosm - Monticello)	selenite added to artificial streams which entered food web and provided dietary exposure	LOAEC for larval edema and lordosis	<18.21[e] (parent tissue)		
Catostomus latipinnis flannelmouth sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab)	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOAEC for survival and growth	>10.2 (larval tissue)	>10.2	>10.2
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001a	dietary and waterborne (lab)	water: selenate; diet: algae exposed to selenate in water then fed to rotifers which were fed to fish	NOAEC for survival and growth	>12.9 (larval tissue)	>23.28	>23.28

Species	Reference	Exposure route	Selenium form	Toxicological endpoint	Chronic value, µg/g dw[a]	SMCV µg/g dw	GMCV µg/g dw
Xyrauchen texanus razorback sucker	Beyers and Sodegren 2001b	dietary and waterborne (lab)	water: site waters; diet: algae exposed to site water then fed to rotifers which were fed to fish	NOAEC for survival and growth	>42 (larval tissue)		
Lepomis macrochirus bluegill	Bryson et al. 1984	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	LOAEC for larval mortality	<59.92[c,d] (parent tissue)	9.50	9.50
Lepomis macrochirus bluegill	Bryson et al. 1985a	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	Chronic value for swim-up larvae	<43.70[c,d] >20.29[c,d] (parent tissue)		
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	NOAEC for swim-up larvae	>5.45[c,d] (parent tissue)		
Lepomis macrochirus bluegill	Gillespie and Baumann 1986	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	Chronic value for larval survival	<28.20[d] (larval tissue); or <21.47[d,e] (parent tissue)		
Lepomis macrochirus bluegill	Coyle et al. 1993	dietary and waterborne (lab)	diet: seleno-L-methionine water: 6:1 selenate:selenite	EC_{20} for larval survival	8.954 (parent tissue - females only)		
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab)	diet: seleno-L-methionine water: 1:1 selenate:selenite	LOAEC for juvenile mortality at 4°C	<7.91 (juvenile tissue)		
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab)	diet: seleno-L-methionine water: 1:1 selenate:selenite	NOAEC for juvenile mortality at 20°C	>6.0[d] (juvenile tissue)		

Species	Reference	Exposure route	Selenium form	Toxicological endpoint	Chronic value, µg/g dw[a]	SMCV µg/g dw	GMCV µg/g dw
Lepomis macrochirus bluegill	Hermanutz et al. 1996	dietary and waterborne (mesocosm - Monticello)	selenite added to artificial streams which entered food web and provided dietary exposure	MATC for larval survival, edema, lordosis and hemorrhaging Study II	12.12 (parent tissue)		
Lepomis macrochirus bluegill	Bryson et al. 1985b	dietary	seleno-DL-cysteine	NOAEC for juvenile growth	>3.74[d] (juvenile tissue)		
Lepomis macrochirus bluegill	Cleveland et al. 1993	dietary	seleno-L-methionine	NOAEC for juvenile survival	>13.4[d] (juvenile tissue)		
Lepomis macrochirus bluegill	Hermanutz et al. 1996	dietary (mesocosm - Monticello)	selenite originally added to artificial streams which entered food web and provided dietary exposure	NOAEC for larval survival, edema, lordosis and hemorrhaging Study III	>17.35[d] (parent tissue)		
Centrarchidae (9 species)	Lemly 1993b	dietary and waterborne (field - Belews Lake, NC)	not determined	EC_{20} for deformities among juveniles and adults	44.57 (juvenile and adult tissue)	NA	NA
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab)	Se-laden shiners from Belews Lake, NC	LOAEC for survival of yearling bass	<14.75[c] (juvenile tissue)	<14.75	<14.75

[a] All chronic values reported in this table are based on the measured or estimated (see footnotes below) concentration of selenium in whole body tissue.
[b] Estimated using the equation III.
[c] Estimated using the equation I.
[d] Chronic value not used in SMCV calculation (see text).
[e] Estimated using the equation II.

National Criteria

The available data for selenium, evaluated in accordance with EPA's guidelines for deriving aquatic life criteria (Stephan et al. 1985) indicate that, except possibly where an unusually sensitive species is important at a site, freshwater aquatic life should be protected if the following conditions are satisfied.

A. The concentration of selenium in whole-body fish tissue does not exceed 7.91 µg/g dw (dry weight). This is the chronic exposure criterion. In addition, if whole-body fish tissue concentrations exceed 5.85 µg/g dw during summer or fall, fish tissue should be monitored during the winter to determine whether the selenium concentration exceeds 7.91 µg/g dw.

B. The 24-hour average concentration of total recoverable selenium in water seldom (e.g., not more than once in three years) exceeds 258 µg/L for selenite, and likewise seldom exceeds the numerical value given by exp(0.5812[ln(sulfate)]+3.357) for selenate. These are the acute exposure criteria. At an example sulfate concentration of 100 mg/L, the 24-hour average selenate concentration should not exceed 417 µg/L.

The available data for selenium, evaluated as above, indicate that saltwater aquatic life should likewise be protected from acute effects of selenium if the 24-hour average concentration of selenite seldom exceeds 127 µg/L. Because selenium might be as chronically toxic to saltwater fishes as it is to freshwater fishes, the status of the fish community should be monitored if selenium exceeds 5.85 µg/g dw in summer or fall or 7.91 µg/g dw during any season in the whole-body tissue of salt water fishes.

Implementation

As discussed in the Water Quality Standards Regulation (U.S. EPA 1983b), a water quality criterion for aquatic life has regulatory force only after it as been adopted in a State water quality standard. Such a standard specifies a criterion for a pollutant that is consistent with a particular designated use. With the concurrence of the U.S. EPA, States designate one or more uses for each body of water or segment thereof and adopt criteria that are consistent with the uses (U.S. EPA 1983c, 1987b). In each standard, a State may adopt the national criterion (if one exists), or an adequately justified state-specific or site-specific criterion.

Criterion concentrations, durations of averaging periods, and frequencies of allowed excursions may be established on a state-specific or site-specific basis (U.S. EPA 1983c, U.S. EPA 1985c). Because the

chronic criterion is tissue-based for selenium, the averaging period only applies to the acute criterion, which is defined as a 24-hour average, based on the speed at which effects may occur in the toxicity tests used for its derivation. Implementation guidance on using criteria to derive water quality-based effluent limits is available in U.S. EPA (1985c and 1987b).