Review of draft, "Aquatic Life Water Quality Criteria for Selenium 2002"
Response to Comments from
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General comments and overall impression of the scientific merit

I am supportive of the approach taken in developing these criteria. Biomonitoring of Se release and potential for chronic impact via fish whole body Se levels represents a reasonable and defendable approach to safeguarding aquatic ecosystems. Pollution is a biological phenomenon and when we measure it in chemical terms we must be able to relate it to any possible negative biological effect. A chronic exposure chemical concentration in an environmental media, such as water or sediment, confounds this relationship because of the required trace element status of selenium in all aerobes and varying degrees of dietary exposure, homeostasis, biotransformation and end effects of various species in a complex ecosystem. The authors have gone to great lengths to be inclusive of the available data and expert opinion in the development of the proposed criteria.

Monitoring of chemical levels in environmental media such as water is usually very satisfying to some because of the precision of the measurements, but this often breaks down when the link to biological phenomena is not available or unclear. There are real challenges in localization of the chemical release and quantifying temporal and spatial variation. With selenium, the complex interplay of inorganic and organic chemical species coupled with diverse biotic and abiotic processes, makes true chemical exposure assessment in a dynamic ecosystem a difficult challenge and in common, non-research, environmental management applications - impossible. Biological systems are "damped" and integrative over time. Hence, receptor monitoring can yield a more accurate assessment of the potential for environmental impact. Spatial variability can still be significant when using organisms and the variability of toxicity among organisms can be great, both within a species and between taxa. However, biologically based monitoring allows for a better ecosystem assessment of migratory populations and real exposure patterns such as concomitant sulfate exposure potentially moderating Se uptake.

The inclusion of an acute water concentration standard adequately recognizes the weaknesses of tissue based monitoring in an acute exposure scenario. Acute disruption of fundamental biological processes and the inability of the organism to overcome the resulting toxicodynamic processes are metabolic in nature and are therefore best quantified by assessment of dose. The acute toxicity of water borne Se to a wide array of aquatic species is well described in the scientific literature. The effects of chronic exposure at low levels in water are confounded by the biogeochemical cycling of Se in aquatic ecosystems and food chain effects that vary considerably with the local environment (e.g. lentic vs. lotic). Management of environmental releases of Se since the 1987 criteria have been difficult and resource intensive due to: 1) limited or conflicting knowledge on site and species specific impacts; 2) unknown field observation variables (e.g.

pesticides and metabolites in Se contaminated agricultural drainage, endocrine disrupting chemicals in Se containing waste water discharges); and 3) limited or non-existent, best available technologies (BATs) to treat Se containing discharge waters to meet the aquatic biota criteria.

Responses to each of the specific questions
Acute criteria in fresh and salt waters

1) Are the toxicity tests used to derive the criteria appropriate for such use? Are you aware of other relevant data that were not used?

Comment: Yes as far as I can tell from the various descriptions. I am not aware of other data.

Response: So noted.

2) Are the acute criteria appropriate?

Comment: Yes. The approach appears to be consistent with EPA guidance and the supporting data appears to be sufficiently comprehensive and complete.

Response: So noted.

3) The criterion did not incorporate a specific relationship with sulfate. However, if there is a need for additional site specific discrimination, are the data indicating a relationship between toxicity and sulfate concentration sufficient to support expressing the freshwater selenate criterion as a function of sulfate concentration?

Comment: Because of the analogous Se:S biogeochemical cycling, the co-location, co-transport, and therefore co-exposure of the elements is of merit in the assessment of acute ecotoxicity. As with most modifiers of toxicity (antagonists, synergists, etc.), much more is known about the primary compound of intoxication rather than the effects of secondary compounds. However, with an ab initio biochemical pathways examination and observations in the literature, most would judge the moderation of Se toxic endpoints by S as well founded. In my work on selenium releases in natural and disturbed environments, I get far more concerned when I observe Se:S ratios in water greater than 1:1000.

A review of the Se:S data compiled in Table 1a show the expected lack of effect for selenite:sulfate exposures. However, the selenate data for most species show a distinct relationship as sulfate levels rise. I would not expect the relationship to be the same in all species (for the same reasons I would not expect a similar color of eyes in all species) and reason #1 (p 15 ¶ 4) uses the rate effect differences inappropriately to justify the action of not adjusting for sulfate. The slopes as calculated in p 15 ¶ 4 (0.19 and 0.87) are inaccurately referred to as "sufficiently mild" in reason #2 for not including a sulfate adjustment (p 15 ¶ 4). Using a lower bounding estimate for the Se:S relationship (0.19?) would be satisfactory to me. Note that using a lower bounding estimate of 0.19 to account for sulfate modification of Se toxicity, a 2000 mg/kg

sulfate brine water would have an adjusted acute criteria of (380 + 185) µg/L = 565 µg/L. This is well below the SMAV of 2,073 µg/L for *H. azteca* and therefore protective. Castle et al. (in preparation) have observed that selenium acute toxicity testing breaks down in sulfate brines as a result of animal desiccation.

As discussed in reason #3 (p 16 ¶ 1), a sulfate correction would not be protective of selenite impacted environments. This is the strongest reason for not maintaining a general sulfate correction to the FAV. One can debate scenarios of the potential for selenite occurrence in natural oxic, high sulfate systems, but it can occur. This is an opportunity for site specific adjustments based on the assay of Se speciation in the system, if EPA decides to go down this path. I would be supportive of this mode of incorporating the sulfate adjustment if it were simple. *For waters with >90% selenate as a fraction of total selenium, an adjusted selenium concentration of 185 + 0.19 [SO4 2-] µg/L is protective of freshwater aquatic life.*

Justification for inclusion of an adjustment may be found in examining the potential applications of the criteria to different use scenarios including sulfate brines. Since the Se:S relationship passes the reasonable and expected judgment most informed scientists would give it, EPA should move forward with developing sulfate guidance for the acute freshwater criterion.

Response: A sulfate correction to the selenate FAV was included in the revised draft document. Appendix A of the revised draft document presents the data and analyses used to determine the correction.

Chronic freshwater criterion

4) Is a concentration in whole body fish tissue an appropriate basis for expressing the criterion?

Comment: Yes. The proposed approach to limiting the environmental impact of low level anthropogenic selenium release employs fish tissue as an indicator of unacceptable risk. This approach is the most direct and uses resident species in the local food chain as a sentinel of threat. Occupying a key position of the food chain of an aquatic ecosystem as well as maintaining independent commercial and recreational value, fish are an excellent choice for monitoring and assessment. Fish whole body Se levels serve alternately as a direct, upper-trophic level dose-response assessment and as an exposure indicator for aquatic birds, especially piscivorus types.

Response: So noted.

5) Is the freshwater chronic criterion appropriate?

Comment: No, not entirely. A weakness of the approach occurs on p 58 when the FCV is lowered on the
basis of a single additive stress study (Lemly 1993a). Up to this point in the discussion, the process was systematic and orderly. Recognition of this study is valid, but additive, synergistic,

potentiated or antagonistic effects as a whole have not been included in the discussion in great detail and certainly not in the FCV calculation. The antagonistic effects of sulfate are explored in the acute criteria development. The potential for beneficial or adaptive effects at low to moderate exposures such as increased immune function, increased growth rates, adaptive enzyme systems for oxidative stress or adaptive biotransformation and elimination observations (thereby increasing tolerance) in naturally exposed populations (specifically excluded in the FCV) are all not quantified in the final development. Species specific responses and cold vs. warm environmental biodynamics of selenium are inadequately treated to justify a cold stress modification to the FCV.

Lemly 1993a appears to be a well conducted study but interpretation of its conclusions should be limited to cold stress on warm water species. Fisheries scientists I have consulted doubt that salmonids would respond similarly and that the bluegill experiment is noteworthy but not definitive when applied to freshwater species as a whole. Indeed, salmonids are biochemically better suited for cold and therefore experience less cold stress. Natural behavior is observed by fish in seeking out a suitable thermocline as a survival response. Given the myriad of potential environmental and biological (species) modifiers to the toxic and beneficial effects of selenium, I would therefore strongly recommend elimination of the results of Lemly 1993a as a modifying datum in the formulation of the criteria. This is especially true in the absence of study replication and the overly broad application of the study interpretation to all fish, cold and warm water species.

It is interesting that the Adams 1976 data from Table 1a presents a different picture of temperature effect on selenium toxicosis. Below, I plot the data from p 20 and demonstrate a negative slope relationship of selenite LD/EC50 with increasing temperature. Although one data set is an acute toxicity trial and the other is a chronic trial, I see the Adams 1976 data as significant in limiting any determination of the Lemly 1993a study as definitive. It is clear that the temperature effects of selenium ecotoxicity are not adequately studied or understood to justify incorporation into the FCV. I have often thought it curious that the 4 ºC increase in Belews Lake temperature as a result of power plant cooling has received only minor attention in addressing its aquatic ecosystem decline. Unless EPA wants to get deep into the game of modifying all similar criteria on the basis of temperature effects, warm and cold, it is best not to invoke it in this singular case on the basis of a single, unreplicated study.

Figure on Temperature Effect on Acute Toxicity of Selenite to Fathead Minnow *Data from Table 1a, Adams 1976*

In regard to the development of the GMCV, I would recommend EPA perform a 3-parameter log normal regression treatment (vide infra) and shown in the spreadsheet attachments: *Muscle to Whole Body Conversion* (page 1-3), *Ovary to Whole Body Conversion* (page 1-3), *Liver to Whole Body Conversion* (page 1-3). This approach will increase the validity of the tissue-whole body Se model derived to calculate the GMCV. Please note that the attached worksheets have not been audited for error and the data and equations developed are shown for information purposes only.

There is a substantial lifespan-through-spawning fish study that is presently concluding and the results should be examined for inclusion into the salmonid GMCV developed in the draft proposal. I have attached a project study report "Effects of dietary selenium on cutthroat trout *(Oncorhynchus clark*i) growth and reproductive performance" by Dr. Ronald Hardy of the University of Idaho (Appendix I). Professor Hardy, a former NMFS researcher, is director of the Aquaculture Research Institute and author of the textbook Fish Nutrition.

This 3-year lifespan study examined fish spawned from fish taken from the Blackfoot River (Se affected watershed) and Henry's Lake (background watershed) near the Western Phosphate Resource Area (WPRA) in and around the Caribou National Forest of South Eastern Idaho. The WPRA has had active mining of phosphate for fertilizer and manufacturing for over 80 years and Se release was first observed in 1996. Additional description of the area, selenium releases and the study are found below and in the attached report. In short, fish from the Blackfoot River (affected) and Henry's Lake (not affected) were examined by molecular biology techniques for genetic differentiation, and thereby survivorship bias and none was found (not shown in this report). Over 6800 eggs from the Blackfoot River fish were examined over two years and the % deformed fry were observed at typical or below levels indicated as normal or background (1999 0.76% and 2000 2.6%, Hardy, Appendix 2). A 2-3 year feeding trial of fish spawned from captured adults was started following assessment of reproductive success. Studies were conducted on Henry's Lake and Blackfoot River cutthroat trout groups using a diet modified by 2, 4, 6, 8, 10 mg/kg Se (Henry's Lake fish) or 5, 10, 15 mg/kg Se (Blackfoot River fish) as selenomethionine (control diet 1-2 mg/kg Se). Growth, feed conversion ratios, Se retention and reproductive success were examined. The spawning for the final group of fish is underway now (May 2002). The update summary reports:

> Groups of Henry's Lake cutthroat trout were fed six experimental diets containing 0-10 mg added selenium as selenomethionine/kg dry diet for 124 weeks (868 days, 2.5 years). In the highest dietary selenium groups, whole body Se concentrations reach a high of 12.5 µg Se/g dry tissue after 44 weeks of feeding, the last Se analysis until spawning. No reduction in appetite, mortality, or difference in size was detected among dietary treatment groups during this period. Fish grew at rates that exceed growth rates of cutthroat trout at state and federal hatcheries. Thus, no effects on fish growth, feed intake, or survival were found when fish were fed levels of dietary selenium, supplied as selenomethionine, as high as 10 µg Se/g diet throughout the entire life cycle of the fish.
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> Groups of Blackfoot River cutthroat trout were troublesome from the beginning of exogenous feeding. No diet formulation developed for rainbow trout, open-formula, experimental, or commercially-available, supported normal growth or health of the fish. Contacts with state and federal agencies revealed that in all situations where cutthroat of wild origin are reared in captivity, the fry were extremely difficult to rear, suffering large losses and poor growth when fed any commercial or agency-specified diet. A completely new diet formulation was developed at the Hagerman Fish Culture Experiment Station, tested for eight weeks, and tested informally at the Jackson Hole National Fish Hatchery (USFWS) on cutthroat fry and fingerlings of wild origin. Results were positive, and the feeding trial with this group of fish was re-started using this diet formulation as the base to which selenomethionine is added. Once the fish reached the post-juvenile stage, they were weaned to the formulation used to rear the Henry's Lake fish (Se level differed, of course).
>
> No signs of toxicity have been observed in Blackfoot River cutthroat after nearly two years of feeding diets supplemented with 0, 5, 10, and 15 µg Se (as selenomethionine)/g diet. Whole body and egg Se levels of

> Henry's Lake fish reflected dietary Se intake. Egg levels were much higher in dietary treatment groups than levels typically observed in eggs of wild fish taken from the Blackfoot River. Thus, the objectives of this study, to orally dose cutthroat trout with the form of selenium found in their food chain and produce fish with a range of intake levels and body levels greater than that found in the Blackfoot River watershed, and to determine the acute and chronic effects of Se intake on growth, feed intake, survival and reproductive performance, have been met, or will shortly be met once egg incubation is completed, and their tissues and eggs are analyzed for Se.
>
> Depuration rates of Se from juvenile cutthroat trout varied with dietary treatment group, appearing to depend upon the whole body level (and body burden) at the onset of depuration. Cutthroat trout containing high concentrations of Se reached approximate baseline levels after 32 weeks of depuration (feeding the control diet). During this period, the fish grew approximately 75%. Growth dilution was insufficient to account for the decrease in Se concentration in the fish, suggesting that Se was excreted, most likely in connection with protein turnover. These results suggest that juvenile cutthroat trout, exposed to high environmental Se levels in the upper sections of the Blackfoot River system, are likely to depurate to much lower levels after leaving upstream nursery areas and migrating downstream to post-juvenile and adult rearing areas, where the major portion of their life cycle is spent and where Se concentrations in the river system are low relative to contaminated areas in upstream tributaries.

This data suggests that cutthroat trout and salmonids in general, may be more tolerant to environmental selenium levels and that a salmonid GMCV may be a more accurate representation of an aquatic life protection threshold in these habitats. The cutthroat trout study will be presented at the Fall SETAC meeting and it is currently in preparation for journal submission.

My suggestions for modifying the current approach are thus:
Calculate a new salmonid GMCV level including the Hardy data as it occurs in the attached report and as complete data are available (Blackfoot River fish) available early this summer. Compare the GMCVs and if the Bluegill GMCV data remains lowest, use it for the FCV. I would encourage a recalculation of the Bluegill GMCV using data obtained from the more statistically rigorous tissue-whole body, 3 parameter log normal regression approach I have shown. This would be a defendable criteria development that avoids species specific levels. A 9.5 mg/kg FCV (Bluegill GMCV) better approaches the draft salmonid GMCV of 11.64 mg/kg and is still protective of aquatic birds.

Response: The issue of setting a criterion based on one study, Lemly (1993a in draft Se document), is a concern to EPA. We considered the best option to be to set the draft criterion to protect juvenile fish based on the conditions of Lemly's test, and retained that approach in the 2004 revised draft. The Lemly study does show a clear effect to juvenile bluegill exposed 60 days to selenium from an aqueous and diet source at 4°C. We found no chronic exposure data for juvenile bluegill contradicting this finding. The trend of decreased toxicity with decreased temperature discussed above is not comparable because of the difference in exposure (aqueous vs. aqueous and diet) and duration of the tests. We believe that the greater sensitivity to sensitive species, such as bluegill, during cold conditions needs to be considered in setting a protective criterion for all species. We recognize cold water species such as salmonids may not show the same increased sensitivity at colder temperatures as did the warm water species, bluegill. However, the GMCV for *Oncorhynchus* in the revised draft document is 10.66 µg Se/g dw,

which is not that distant from the 7.91 µg Se/g dw value.

EPA recognizes the limitations of setting the criterion based on a single study having a single exposure concentration (other than the control), and a temperature regime that may be realistic for some parts of the country but not for others. Although the revised draft document states that results from appropriate site-specific studies could be used to modify the criterion to account for differences between aquatic systems, we recognize that in practice that would not be easy to implement in the absence of an EPA protocol.

Recognizing the importance of the issues raised in the comment, EPA is asking the public for scientific information, data, and views on the use of the Lemly study results and on ways to incorporate climate- or ecosystem-dependent site-specificity into the criterion.

6) With the goal of being neither under- nor overprotective, how reliable would you expect the criterion to be in application to different sites? Are there any straightforward ways of improving its site specificity?

Comment: The criterion is intended to safeguard natural resources. The presented data suggest that an FCV of 9.5 mg/kg (Bluegill GMCV) would be sufficiently protective based on the preponderance of current data. Invocation of temperature effects via the Lemly 1993a study into the criteria development complicates the application across cold and warm water species. Unless EPA desires to develop species specific criteria, temperature effects are best not included in the FCV.
The 9.5 mg/kg fish tissue value better approaches the salmonid GMCV (cold water fishes) and therefore is directly applicable to a wider range of sites. A 9.5 mg/kg FCV balances the available data consistent with current practice. We do not currently apply warm water stresses to cold water fishes in application of chemical water quality standards. Application of a cold stressed, warm water species FCV is likely to cause concern in the Western and Northern part of the US where the environment and resident species are significantly different.

In the discussion of potential ecosystem impacts of various FCV whole body fish Se levels, one needs to keep track of wet weight and dry weight representations – too often a source of confusion. Food, and therefore selenium, is presented in nature as wet weight. It is very important to note that the wet weight transformation of a 9.5 mg/kg dry weight FCV, calculated with the EPA 80% moisture correction (p 46), yields a whole body wet weight level of 1.9 mg/kg. This number is in the range of commercially produced fish chows and the range of the basal diet fed to the control fish in the attached Hardy study (1-2 mg/kg)[1]. The Lemly 1993a study used a Tetramin® control feed with 0.8 mg/kg selenium. This has equally important considerations in the projection of a 9.5 mg/kg dw FCV to ecological risk assessment and food chain effect. The attached work of Hardy (Table 11) shows that a cutthroat trout whole body Se

[1] The fish diet Se value, 1-2 mg/kg is a dry weight value. The moisture content of the feed by proximate analysis was 6.3% (Hardy Table 1). A wet weight value is substantially the same.
[2] Calculated from 8.0 mg/kg selenomethionine added to a 1.5 mg/kg basal diet concentration.

level of 9.37 ± 4.67 mg/kg dw is attained after 2.5 years of dietary exposure at 9.5 mg/kg Se in the feed[2]. This analysis suggests that concerns should be minimal and an FCV value of 9.5 mg/kg dw is protective.

I have concerns that overly protective selenium criteria can venture into the realm of the adaptive response of the antioxidant enzyme system that includes Se-glutathione peroxidase. In work examining liver Se relationships with other metals, I developed the hypothesis that oxidative stress could cause an increase in bulk hepatic tissue and body burden Se levels (Möller 1996) and this could contribute to the observation of selenium problems in multiply contaminated zones such as agricultural drainage ponds. One of my students completed a 30 day, randomized block, static replacement pilot study with juvenile Fathead minnows. All fish were fed commercial trout chow that had background 1 mg/kg total Se. The water for treatment fish contained the herbicide Paraquat, a redox cycling compound understood to cause oxidative stress. The table below shows the whole body Se levels increased 128% compared to the controls. Increases were also noted for iron and manganese, metals also involved in the antioxidant enzyme system. These pilot study results suggest the potential for Se ecosystem effects that are unrelated to Se release. Selenium (Hoffman et al. 1998a, 1998b) itself has been identified as an oxidative stress inducing compound and thus joins the ranks of other NADH reducible metals, complexes and organic molecules that can induce this effect in organisms. We are presently developing a total set of enzyme, antioxidant and free radical assays to take this study into a formal phase.

Fathead minnow: whole body antioxidant metal increase with Paraquat (50 µg/L) exposure (n= 5 groups).

mg/kg dw	Control	Paraquat Treated	% Increase	p Value
Se	0.53	1.21	128	0.27
Mn	12.8	16.2	27	0.10
Fe	142	619	334	0.23

Site specificity of the criteria may be enhanced by commenting on population level concern. Lotic aquatic systems often have confined populations whereas lentic systems are often migratory. There have been several observations of varying degree of impact in lentic vs. lotic Se exposure and this may be the result of the in-migration and out-migration behaviors of the respective populations. The major chronic toxicity endpoint of concern for selenium is reproductive failure. You will not find many biologists that will disagree that reproductive failure is a population level concern. Inserting a population reference will prevent the observation of one fish with whole body Se exceeding the FCV from being interpreted as an indicator of ecosystem collapse.

There is significant regulatory guidance concerning population level concerns in environmental management:

"ecological effects of most concern are those that can impact populations (or higher levels of biological organization)." (USEPA 1997).

"Superfund remedial actions generally should not be designed to protect organisms on an individual basis (the exception being designated protected status resources, such as listed or candidate threatened and endangered species or treaty-protected species that could be exposed to site releases), but to protect local populations and communities of biota." (USEPA 1999).

The ecological entity to be protected "can be a species (e.g., eel grass, piping plover), a functional group of species (e.g., piscivores), a community (e.g., benthic invertebrates), an ecosystem (e.g., lake), a specific valued habitat (e.g., wet meadows), a unique place (e.g., remnant of native prairie), or other entity of concern." (USEPA 1998).

The biogeochemical cycling of selenium in aquatic ecosystems makes population concerns important. I would recommend inserting into the criteria the sentence:

"The potential for reproductive failure in selenium exposed organisms makes population level protection important."

Response: The comments on the lack of effects to cutthroat trout that suggest a FCV of 9.5 µg/g dw is so noted, as is your hypothesis and observation on the effect of liver tissue-inducing chemicals on selenium body burdens. The comments pertaining to EPA's objective to protect populations is correct and we are in agreement that language to that effect should be added to the document.

At some point in the future, EPA intends to make use of population models within the criteria program. But we currently have no specific plan either to use or not to use population models during development of the final selenium criterion.

Finally, the comment touches upon the issue of what constitutes exceedance of the criterion when tissue concentrations among sampled individuals at a site vary, some above, some below the criterion. That issue will be taken up during preparation of the implementation guidance.

7) Although the criterion was not derived using wildlife criteria derivation procedures, EPA noted some evidence that the criterion would protect piscivorus birds. Are you aware of other data relevant to the protectiveness of the criterion for birds?

Comment: Opresko et al. (1995) developed dietary selenium thresholds for piscivorus birds using mallard toxicity data for selenite (Heinz et al. 1987) and selenomethionine (Heinz et al. 1989). Selenomethionine most closely resembles actual diets. Heinz et al. (1989) exposed mallards to selenomethionine fortified feed and evaluated reproductive success and hatchling survival. The NOAEL and LOAEL for reproductive impairment observed 4 and 8 mg/kg. Opresko et al. (1995) estimated dietary selenium thresholds for these piscivorus birds using the ingestion rate and body weight for mallards reported in Heinz et al. (1989) and species-specific ingestion rates and body weights for piscivorus birds (belted kingfisher, great blue heron, osprey). As shown in the draft criteria document (p 60), these dietary thresholds ranged from

10.6-12.2 mg/kg, suggesting that a fish tissue-based criterion of 9.5 mg/kg (Bluegill GMCV) would be protective of piscivorus birds.

The chronic toxicity of selenomethionine to the piscivorus black-crowned night heron was evaluated by Smith et al. (1988) in a 94 day reproductive study. Their work observed a dietary NOAEL of 10 mg/kg based on reproductive effects. Thus, a whole body fish tissue criterion of 9.5 mg/kg is less than the chronic NOAEL for this piscivorus bird.

The trophic transfer of selenium from food to bird egg was analyzed in field-collected data for several species of birds at 15 sites in the Western U.S. (Adams et al. 1998). This work suggests that trophic transfer is much less in the field than in the laboratory studies performed by Heinz et al. (1989). The field data indicate that on average the trophic transfer is 1.1 while the laboratory study indicates trophic transfer factors of 2-4 from food to mallard duck eggs suggesting a positive bias of the laboratory determined rate vs. the field determined rate[3]. Additionally, by research design, the field study integrates species variability, genetic differentiation and food Se speciation diversity into the development of a food to bird egg trophic transfer rate. A high trophic transfer rate in the laboratory study of Heinz et al (1989) is not unexpected since selenomethionine, the dosing agent in this study is more actively incorporated into tissue than selenite, selenate or selenocysteine (Burke, 1986), all of which would be components of a natural diet in varying proportions. Use of the 1.1 trophic transfer factor to assess selenium transfer from food to bird eggs indicates that at a dietary concentration of 9.5 mg/kg would yield a bird egg concentration of 10.45 mg/kg. This level is below the calculated concern thresholds of 16 mg/kg (Fairbrother et al. 2000), 12-15 mg/kg (Adams et al. 2002 In Preparation) and 12.8 mg/kg (Ohlendorf 2002, in press). It is above an earlier 6-8 mg/kg conservative threshold suggested by Skorupa et al. (1996) in a U.S. Fish and Wildlife Service guideline. The preponderance of work in this area suggests that a 9.5 mg/kg FCV (developed from the Bluegill GMCV) would be protective of birds.

A large population-scale study of avian selenium effects in the selenium contaminated WPRA ecosystem is in it final stages. The study is being conducted by Professor John Ratti and Professor Edward Garton of the Wildlife Resources Department of the University of Idaho. The project has evolved from an egg study to a nesting and reproductive success study. In 1999 and 2000, approximately 250 and 350 eggs were collected, respectively, representing about 20 species. Seven nesting success indicators were greater on mining impacted sites and eight nesting success indicators were greater on background sites, allowing limited differentiation of reproductive success in the two environments. In 2001, the project attempted to use four species for reproductive success studies consisting of the American Robin, Red-Winged Blackbird, Coot and Yellow-Headed Blackbird. Approximately 450 eggs were collected but because of the low water year, only the robin and red-winged blackbird could be represented in the study. The study measured hatching and fledging success using a significant number of nesting sites that

[3] For comparison, the average selenium food to egg transfer factor in the Hardy Cutthroat trout study was 1.2 (hardy, Table 11).

represented background and mining disturbed areas. The researchers conducted a stratified random sample of aquatic/riparian habitat patches for the entire study area incorporating sampling strata based on a combination of National Wetland Inventory polygons and mining vs. reference regions. They used complete counts of 57 sites to determine bird abundance, total number of nests started and nest success (both hatching and fledging) for 4 species (red-winged blackbirds, yellow- headed blackbirds, American coots and American robins). The field study found more than 600 nests but droughty conditions limited abundant nest data only for red-wing blackbirds (aquatic) and robins (terrestrial).

The field research teams took one egg from each nest for Se analysis and monitored nest activity over the season. With this data, logistic regression can be used to evaluate how hatching success and fledging success decreased with increasing Se levels. More than 45% of the eggs had Se levels above 5 mg/kg and many were above 12 or 16 mg/kg, levels that have been identified in the literature as significant in exposure and risk (vide supra). However the surprising result was that all of the logistic regressions for hatching success and fledging success in both red-wings and robins showed positive slopes for egg Se concentration. In all cases increased Se levels in bird eggs were associated with higher levels of nesting success. Likewise the field teams found not a single case of terata in more than 1000 eggs and fledglings examined from over 20 species. The investigators expected that reproductive success would start to decline at very high levels of Se but the nests with the highest levels of Se (around 30 mg/kg) both hatched and fledged young successfully. The researchers have hypothesized that the observed beneficial effect of selenium exposure may be a result of the migratory behaviors of the WPRA nesting bird populations throughout the largely marginal or deficient Se areas of the West and Northwest areas of North America.

These results, in light of the observations at Kesterson and elsewhere, suggest that birds may have variable responses to Se exposure and therefore are poor candidates as sentinel indicators. The migratory behaviors of many birds may limit opportunistic exposure to isolated selenium contaminated zones. Indeed, some feeder fish sampled from the primary contaminated areas of the WPRA exceed the proposed whole body Se criteria. Primary Se release sites have demonstrated water Se levels in excess of 2 mg/L and some secondary waters show significant exceedances of the current 5 µg/L Se criteria. Yet, avian population modeling at the site indicates that if there was a “magical” conversion of mine sites into background sites (i.e. the population dynamics, including reproductive success, of mined areas were substituted into the model for background sites) no population level change would occur. Twenty year bird population modeling in this study shows stable populations. This suggests that a satisfactory level of protection is afforded birds under the proposed criteria approach. The WPRA bird studies will be presented at the Fall SETAC meeting and they are currently in preparation for journal submission. Additional Spring 2002 nest surveys are underway.

Response: In accord with an agreement with the USFWS, the section on birds has been deleted in the revised draft document.

Specific comments for recommended changes needed to improve the clarity and scientific

accuracy of the document

Comment: The document uses µg/g as the concentration unit. Consider using the preferred SI unit mg/kg.

Response: The unit used for organic residues in other AWQC documents is mg/kg. We are unsure why µg/g is some commonly found in the selenium technical literature. For the 2004 draft we retained µg/g, but we recognize that the micro symbol is a nuisance.

Comment: p 2 ¶ 1: It is doubtful that "substantial" concentrations of Se(II) are ever found in oxygenated alkaline waters. USEPA 1987a is a meta-analysis and a weak reference for this.

Response: Reviewer is correct with respect to inorganic Se(II). However considerable amounts of Se(II) in organic form can be encountered. Last sentence of paragraph 1 will be changed to read: "Substantial concentrations of both selenium(II) in organic form and selenium(IV) are not uncommon (Cutter, 1989; Sappington, 2002)." The 1987 reference will be deleted and two different ones inserted.

Comment: p 2 ¶ 2: Please provide a reference for the last sentence.

Response: Several references will be added. A substantial portion of selenium in surface waters may exist in organoselenium forms or complexes (Lahermo, 1998; Zhang, 1996).

Citations for added references:

Cutter, G.A, "Freshwater systems". Ihnat M., (Ed.) Occurrence and Distribution of Selenium, (1989), pp. 243-262 CRC Press, Boca Raton, FL.

Sappington, Keith G., 2002, Development of aquatic life criteria for selenium: a regulatory perspective on critical issues and research needs, Aquatic Toxicology, 57 (1-2): 101-113.

Lahermo, P., Alfthan, G. and Wang, D. 1998. Selenium and arsenic in the environment in Finland, J. Environ. Pathol. Toxicol. Oncol. 17 (3-4): 205-216.

Yiqiang, Z. and J. N. Moore. 1996. Selenium fractionation and speciation in a wetland system. Environ. Sci. Technol. 30: 2613-2619.

Allan, C.B., Lacouriciere, G.M. and Stadtman, T.C. 1999. Responsiveness of selenoproteins to dietary selenium. Annu. Rev. Nutr. 19: 1-16.

Comment: p 2 ¶ 3 line 3: "uncontaminated" is an awkward, inaccurate descriptor. Try: non-seleniferous.

Response: Noted. Sentence was changed to read: "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)."

Comment: p 2 ¶ 3 line 5: Try alkaline rather than "drier".

Response: Noted. Sentence was changed to read: "It is abundant in the alkaline soils of North America from the Great Plains."

p 2 ¶ 3 line 11: Delete "in high concentrations" – these are imprecise words.

Response: Noted: Sentence was changed to read: "In addition, selenium occurs naturally in coal and fuel oil and is emitted in flue gas and in fly ash during combustion."

p 3 ¶ 2 line 3: Try biosynthesis rather than "manufacture".

Response: Noted. Sentence will be changed to read: "Selenium is an essential element required as a mineral cofactor in the biosynthesis of glutathione peroxidases."

p 3 ¶ 3 line 3: Delete "the damaging (oxidizing)".

Response: Noted. Sentences were changed to read: "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."

p 2 ¶ 3 line 14: continuing p 4 ¶ 1 line 2, 3: This is a broad overstatement. Nutritional research has demonstrated variable uptake - some homework needed here.
From the attached Hardy study (p 3-4):

> The biological availability of selenium for fish differs with selenium source. Bell and Cowey (1989) reported that the selenium present in fish meal has a low availability to rainbow trout, while that of selenomethionine is high. Lorentzen et al. (1994) observed differences in bioavailability between selenite and seleno methionine on the basis of muscle and whole-body selenium concentrations. Fish fed diets supplemented with selenomethionine had 3-5x higher muscle selenium levels than fish fed equivalent dietary selenium levels, with sodium selenite as the supplement. Studies of bioavailability are principally focused on avoiding selenium deficiency by taking into consideration the bioavailability of selenium from various dietary sources.

Response: So noted.

p 44 ¶ 2, 3; p 45 Figure 4; p 46 ¶ 1: EPA must correct an error that occurs in Figure 2 on p 45 and in equation II found on page 46. In checking the data from Appendix G for development of equations that relate muscle, ovary and liver Se concentrations to whole body concentrations, I

find an error in the determination of equation II. The regression statistics developed for the ovary conversion are incorrect. The corrected analysis, $[Se]_{whole\ body} = 0.45[Se]_{ovary} + 1.32$, is shown in the figure below. A spot check of the data (p H-21) calculated by the equation below yielded a correct result and this suggests that the error is a typo and was not propagated through the calculated tissue conversions. It appears that the r^2 value was inadvertently substituted for the slope value in the written equation. However, ordinary linear regression is not the best approach.

Figure on Se in whole body vs. ovary

The tissue conversion approach shown by EPA outlines a method to increase the amount of data available for whole body Se levels vs. effects from the cited literature using linear regression. Although the relationships are clear, a review of the statistical approach used may offer alternative modes of analysis that will increase the rigor of this operation and the subsequent use of the modified data in the calculation of effects relationships. Specifically, many biostatisticians may expect to see the regression be a log-log equation especially since the data range exceeds an order of magnitude. Confirming this is the heteroscedasticity (non-uniform variance) of the scatter about the regression line which gets larger as the concentrations get larger. To correct for this I worked with a biostatistician colleague on a possible alternate approach to model the relationship between whole body fish Se concentrations and muscle, ovary and liver concentrations. Descriptions of the approach basics are found in Helsel and Hirsch (1992) and similar statistics texts.

The results of this effort are found in the attached spreadsheets: *Muscle to Whole Body Conversion* (page 1-3), *Ovary to Whole Body Conversion* (page 1-3), *Liver to Whole Body Conversion* (page 1-3). In these spreadsheets, the muscle, ovary or liver tissue Se data is tested for lognormality and goodness of fit (page 1). The whole body data is similarly tested (page 2). The estimated lower bound of the tissue values was found by optimizing the r^2 of the fit plot regression[4]. On page 3 of the spreadsheets, I show the 3 parameter lognormal plot and regression equation as well as the 2 parameter normal plot and regression equation.[5] In all cases (muscle, ovary and liver) the goodness-of-fit statistics are better for the 3 parameter log normal regression model. In the third graph of page 3 in each series, I examine the assumption of no difference between species that is implicit in the development of the equations I, II, and III on page 46 of the draft criteria document. For the muscle analysis, I find satisfactory support for the assumption. In the case of ovary and liver, this assumption is weaker. Examination of the trout liver values compared to the bluegill and bass demonstrates significant separation of the observations. The possible explanations for the apparent difference are many and include trout having an enhanced Se hepatic biotransformation and elimination efficiency. However, given the

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[4] Optimizing the r^2 was accomplished by plotting the standard normal variate (adjusting for ties) on the x-axis against ln(x-l) on the y-axis, changing l until r^2 was maximized. This was done using Excel's RSQ(ARRAY 1, ARRAY 2) function, with ARRAY 1 being z and ARRAY 2 being ln(x-l).

[5] The exploratory calculation for regressions of the 2nd kind was performed on another spreadsheet and is not shown.

small number of observations, separating trout data on the basis of species difference is probably not justified in the current treatment.

The results of the analysis are thus:

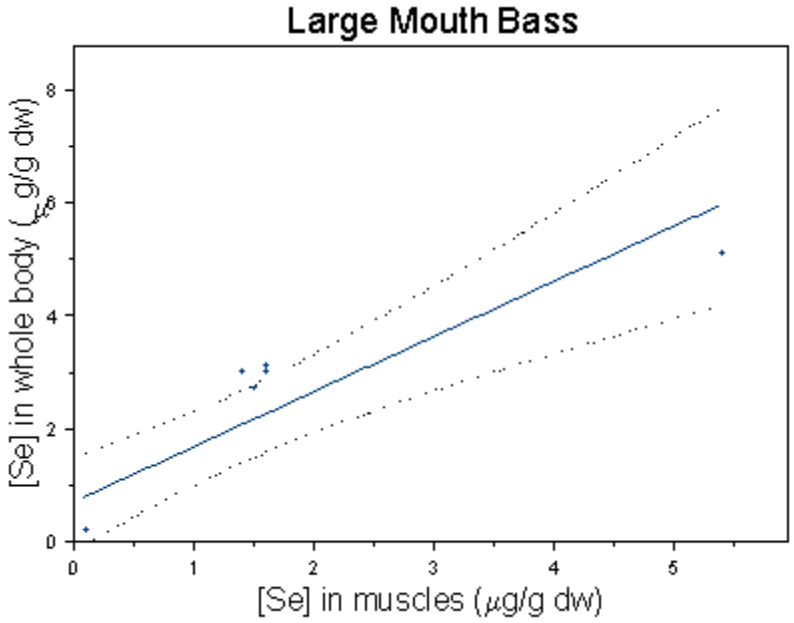
- The ordinary least squares regression presented by EPA is invalid.
- All of the data (whole body, muscle, ovary, and liver) are log normally distributed.
- A 3-parameter, log normal regression represents a more accurate representation of the tissue-whole body Se relationships.
- Batching different fish species in the data sets is satisfactory for muscle, but less so for ovary and liver.
- The modeled numerical relationships between whole body and tissue specific Se concentrations are:

$[Se]_{whole\ body} = 1.63\ ([Se]_{muscle} + 0.78)^{0.80} - 1.23\ r^2 = 0.96\ (r = 0.98)$
$[Se]_{whole\ body} = 1.57\ ([Se]_{ovary} + 0.74)^{0.68} - 1.08\ r^2 = 0.85\ (r = 0.92)$
$[Se]_{whole\ body} = 0.24\ ([Se]_{liver} + 2.47)^{1.04} - 0.67\ r^2 = 0.78\ (r = 0.88)$

Response: Reviewer Greg Moller reported several problems in our regression analyses, which estimated selenium concentrations in the whole body as a linear function of selenium concentrations in liver, ovaries, or muscles. Following his recommendation, we have corrected an error in Fig. 2, page 45: the written equation did not correspond to the regression line drawn. Greg Moller criticized the use of data from multiple species because of apparent differences in slopes of regression lines between taxa. We did not perform an analysis of covariance to test for such differences. The objective of including data from multiple species was to increase sample size and to estimate slopes and intercepts of regression lines for multiple taxa, including those for which very few data were available. Most of the studies we reviewed measured concentrations of selenium in bluegill tissues. For this species, we have a large number of samples, but for all others, the restricted sample size severely limit our ability to infer adequacy of the linear model. For instance, consider the data for large mouth bass (Fig. 1):

$y = 0.68 + 0.98x$
$r^2 = 0.83$

Figure 1. The regression line, fitted by least squares, estimates average selenium concentrations in the whole body of large mouth bass as a function of selenium concentrations in muscles. Dotted lines represent the 95% confidence interval on projected response values.



There are only 9 points to estimate the regression, of which 4 are identical (0.1, 0.2). With such low sample size there is great uncertainty about the shape of the relationship between selenium concentrations in the whole body and in muscles. Inspection of residuals (Fig. 2) suggests that a curvilinear relationship would be more appropriate, yet if additional points were available, it is possible that the distribution of residuals would become symmetrical around zero. Independent regression analyses for each species would likely improve the coefficient of determination (r^2) for most taxa, but confidence intervals for species with low sample sizes would certainly broaden. For example, compare the width of confidence intervals in figures 1 and 3.

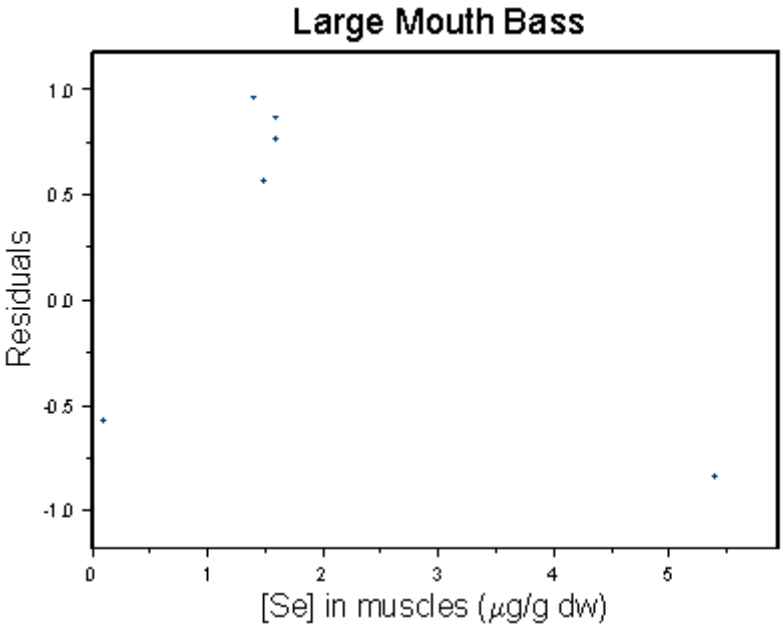


Figure 2. Plot of residuals (y_i - $\bar{y}$) versus concentrations of selenium in muscle tissues of large mouth bass.

Given the low number of samples available for largemouth (*n*=9), tilapia (*n*=1), and carp (*n*=1), we argue that a pooled regression line is a more efficient use of the data, even if it ignores potential differences in slopes among species. In fact, given our sample size for tilapia and carp it is not even possible to perform a regression analyses for these taxa.

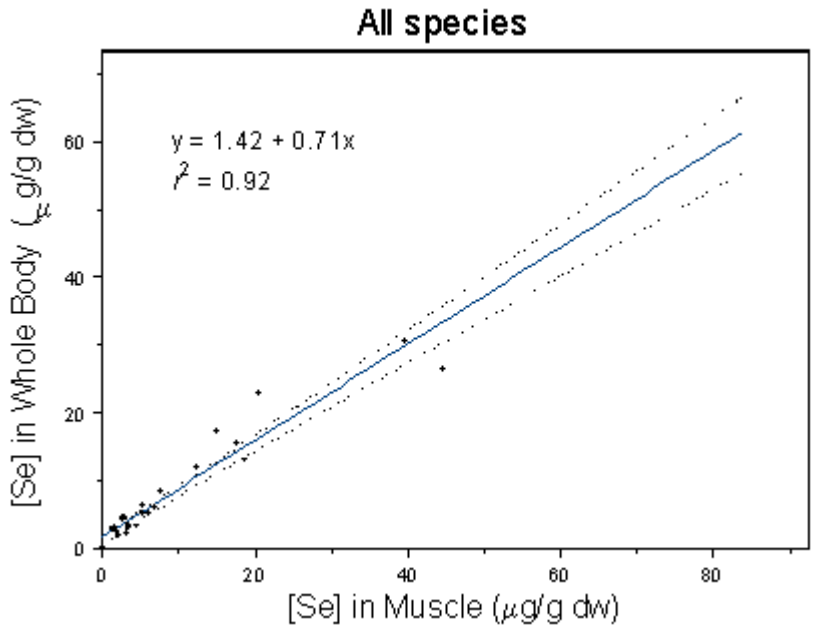


Figure 3. The regression line, fitted by least squares, estimates average selenium concentrations in the whole body of bluegill, large mouth bass, tilapia and carp as a function of selenium concentrations in their muscles. Most data are from bluegill. Dotted lines represent the 95% confidence interval on projected response values.

Table 1. Tests for homogeneity of variance in residuals of regressions modeling average selenium concentrations in the whole body as a linear function of selenium concentrations in selected tissues. Each *F* ratio was computed as the quotient between error mean squares in the second and first halves of observations, sorted by concentration of selenium in tissue.

Residuals of regression	*F*	*P*	df/df
Liver [Se] × Whole body [Se]	33.49	<0.001	20/20
Muscle [Se] × Whole body [Se]	25.24	<0.001	16/17
Ovary [Se] × Whole body [Se]	8.41	<0.001	17/18

We used quantile regression to estimate median concentrations of selenium in the whole body as linear functions of selenium concentration in selected tissues (Figs. 4-6). Quantile regression fits a line to the data such that a selected proportion t (the quantile) of observations are below and the complementary fraction $1-t$ are above it (Koenker and Basset 1978). Estimates of model parameters minimize the sum of absolute deviations. In contrast, ordinary least squares minimize the sum of squared deviations. Least absolute deviation is less sensitive to outliers than least squares (Birkes and Dodge 1993). Other desired properties of quantile regression include: it is equivariant to scale changes, location shift, and monotonic transformations (Koenker and Basset 1978, Koenker and Portnoy 1996). Furthermore, with rank-score statistics it is possible to test hypotheses and build confidence intervals for model parameters with heteroscedastic errors (Koenker 1994, Koenker and Machado 1999). The rank-score test does not have to assume homogeneous error distributions because the statistic is based on signs of residuals and not their size (Koenker and Machado 1999). For introductory presentations of quantile regression see Cade et al. (1999) and Koenker and Hallock (2001). All quantile regressions reported here were performed using the R software (Ihaka & Gentleman 1996) version 1.6.0.

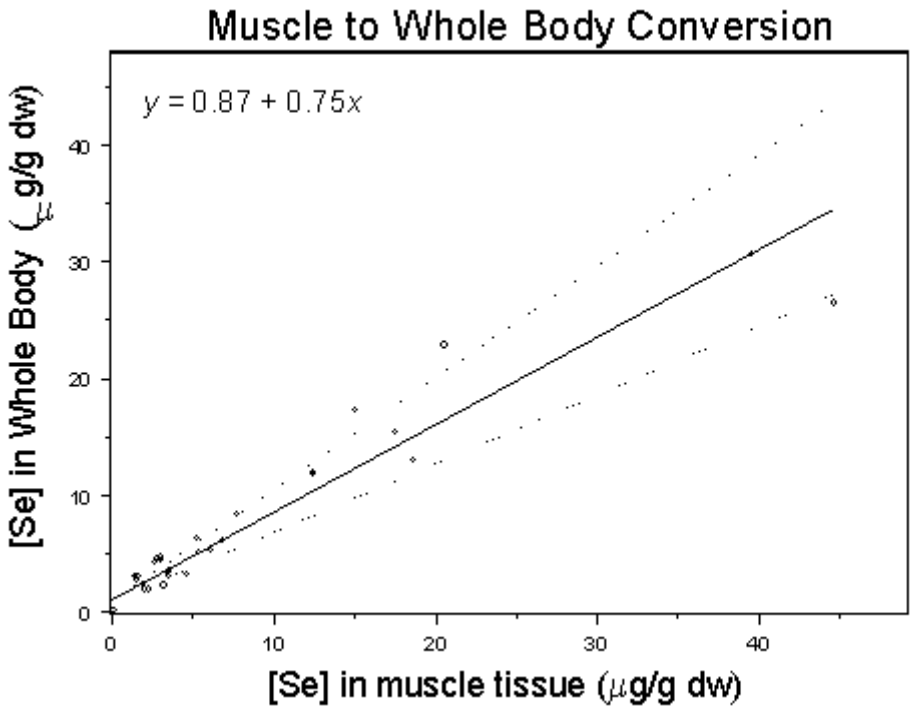


Figure 4. The quantile regression line, fitted by least absolute deviations, estimates median selenium concentrations in the whole body of bluegill, large mouth bass, tilapia and carp as a function of selenium concentrations in their muscles. Most data are from bluegill. Dotted lines represent the 95% confidence interval on projected response values.

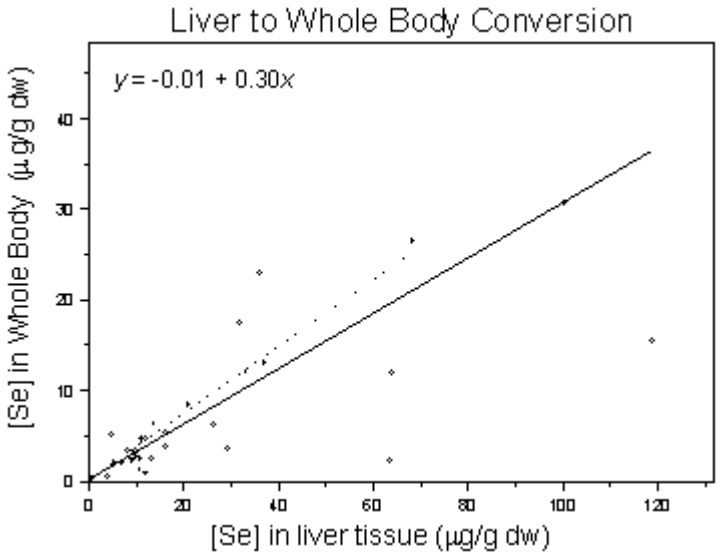


Figure 5. The quantile regression line estimates median selenium concentrations in the whole body of bluegill, large mouth bass, tilapia and carp as a function of selenium concentrations in their liver tissues. Most data are from bluegill. The dotted line represents the upper bound of the 95% confidence interval on projected response values. The lower bound is not displayed because computed values are negative

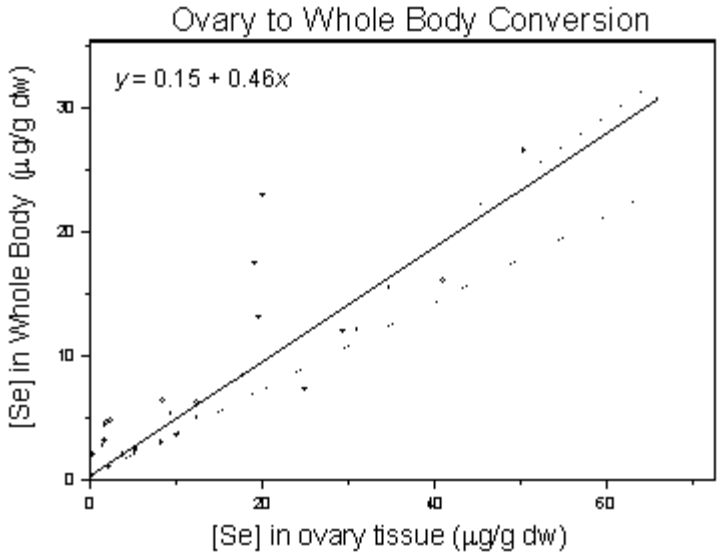


Figure 6. The quantile regression line, fitted by least absolute deviations, estimates median selenium concentrations in the whole body of bluegill, large mouth bass, tilapia and carp as a function of selenium concentrations in their ovary tissues. Most data are from bluegill. Dotted lines represent the 95% confidence interval on projected response values.

Finally, Greg Moller recommended the 3-parameter lognormal regression because it provided a better fit to the data than the linear and 2-parameter lognormal model. Addition of a new parameter usually does not increase the sum of deviations from model projections. The improved fit, though, has to be sufficiently large to justify the extra parameter. Otherwise, they would be sequentially incorporated into the model until its projections exactly matched the observed data. Models would be exceedingly complex and would not help to elucidate the most important factors controlling a system or the basic relationship between variables. An objective approach to select models is described by Burnham and Anderson (2002). It is based on the Kullback-Leibler information, $I(f,g)$, which expresses the information lost when model g is used to estimate the full reality (f). Obviously, the full reality is never known, but $I(f,g)$ can be estimated by the Akaike Information Criterion (AIC, Akaike 1973)

$$AIC = -2 \log(\mathcal{L}(\text{parameters}|\text{data})) + 2K$$

where K is the number of parameters in the model and $\mathcal{L}(\text{parameters}|\text{data})$ is the maximized likelihood of parameter estimates for the available data.. The AIC is a poor estimator of $I(f,g)$ when $n/K < 40$ (n is the sample size). In such instances, a second-order version of AIC, AIC_c, is recommended (Hurvich and Tsai 1989):

$$\mathbf{AIC}_c = \mathbf{AIC} + \frac{\mathbf{2}K(K+\mathbf{1})}{n-K-\mathbf{1}}$$

The AIC and AIC_c are used to rank candidate models. Comparisons among the M ranked candidates are based on the Akaike weight (w), which represents the likelihood of a model given the data

$$w_i = \frac{\exp\left(\frac{-\Delta_i}{2}\right)}{\sum_{m=1}^{M}\exp\left(\frac{-\Delta_i}{2}\right)}$$

where Δ_i is the difference in AIC (AIC_c) between model i and the model with the lowest AIC (AIC_c) value. Weights for all candidate models sum to 1.

We considered three candidate models to project selenium concentrations in the whole body ($[Se]_{WB}$):

I) $[Se]_{WB} = a$.

II) $[Se]_{WB} = a + b\,[Se]_{Tissue}$ and

III) $[Se]_{WB} = \exp(a + b \log([Se]_{Tissue}))$

where a and b are the model parameters we wish to estimate. Model (I) implicitly assumes that selenium concentrations in the whole body are independent of selenium concentrations in liver,

muscle, or ovary tissues. Model (II) projects selenium concentrations in the whole body as a linear function of selenium concentrations in a tissue. Model (III) estimates selenium concentrations in the whole body as an exponential function of the logarithm of selenium concentrations in a tissue. This model is derived from the recommended log-log regression,

$$\log([Se]_{WB}) = a + b \log([Se]_{Tissue}).$$

Back transformation of the response variable to a linear scale is necessary to compare results with the other two models, and to compare model projections with the appropriate criterion. For each model, we computed the sum of weighted absolute deviations (SWAD), AIC_c and the Akaike weight (Table 2). Hurvich and Tsai (1990) demonstrated that the modified version of AIC_c for least absolute deviation ($L1AIC_c$) provides an unbiased estimator for the Kullback-Leibler information, but the small sample criterion for normal least squares regression, which is less computationally demanding, performs equally well

$$\mathbf{AIC} = n\log(\sigma^2) + 2K$$

where σ^2 is estimated as the sum of squared residuals divided by n. For the least absolute deviation regression, σ^2 is estimated as $(SWAD/n)^2$, thus AICc is computed by the expression

$$\mathbf{AIC} = 2n\log(\mathbf{SWAD}/n) + 2K\left(\frac{n}{n-K-1}\right)$$

Table 2. Weight (w), rank, and coefficient of determination (R^1) for candidate models

Tissue: Muscle (n = 21)

Model	k	SWAD	AICc	Delta	Weight	Rank	R^1
$[Se]_{WB} = a$	2	66.00	52.76	59.20	1.27e-13	3	
$[Se]_{WB} = a + b\,[Se]_{Tissue}$	3	16.84	-1.85	4.59	9.17e-02	2	0.74
$[Se]_{WB} = \exp(a + b*\ln([Se]_{Tissue}))$	3	15.10	-6.43	0.00	9.08e-01	1	0.77

Tissue: Ovary (n = 23)

Model	k	SWAD	AICc	Delta	Weight	Rank	R^1
$[Se]_{WB} = a$	2	73.95	58.32	46.89	3.31e-11	3	
$[Se]_{WB} = a + b\,[Se]_{Tissue}$	3	25.20	11.46	0.03	4.97e-01	2	0.66
$[Se]_{WB} = \exp(a + b*\ln([Se]_{Tissue}))$	3	25.18	11.43	0.00	5.03e-01	1	0.66

Tissue: Liver (n = 26)

Model	k	SWAD	AICc	Delta	Weight	Rank	R^1
$[Se]_{WB} = a$	2	41.05	28.27	22.81	1.11e-05	3	
$[Se]_{WB} = a + b\,[Se]_{Tissue}$	3	25.20	5.46	0.00	9.99e-01	1	0.39
$[Se]_{WB} = \exp(a + b\ln([Se]_{Tissue}))$	3	40.83	30.56	25.10	3.54e-06	2	0.01

The linear model (II) was selected the best among the three candidate functions for projecting concentrations of selenium in the whole body as a function of selenium concentrations in the liver (Table H-1). The exponential model (III) was selected the best for projections based on

concentrations of selenium in muscles and ovaries. However, fits of models II and III to ovary data had similar weights. As the best model may not explain much of the observed variation in the data, we calculated coefficients of determination (R^1), defined as

$$R^1 = 1 - (SAF/SAR)$$

where SAF and SAR are the sum of weighted absolute deviations for the full and reduced models, respectively (Cade and Richards 1996). Coefficients of determination for models II and III were also very similar, suggesting that both models are equally effective in predicting concentrations of selenium in the whole body as a function of selenium concentrations in ovaries. With such knowledge, we opted to use the linear model (II) because it is easier to compute. The exponential model for muscle presented the highest coefficient of determination (0.77), indicating that samples of selenium concentrations from this tissue are more effective predictors than samples from liver and ovaries. The fitted quantile regression curves

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

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

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

are shown in figure 7.

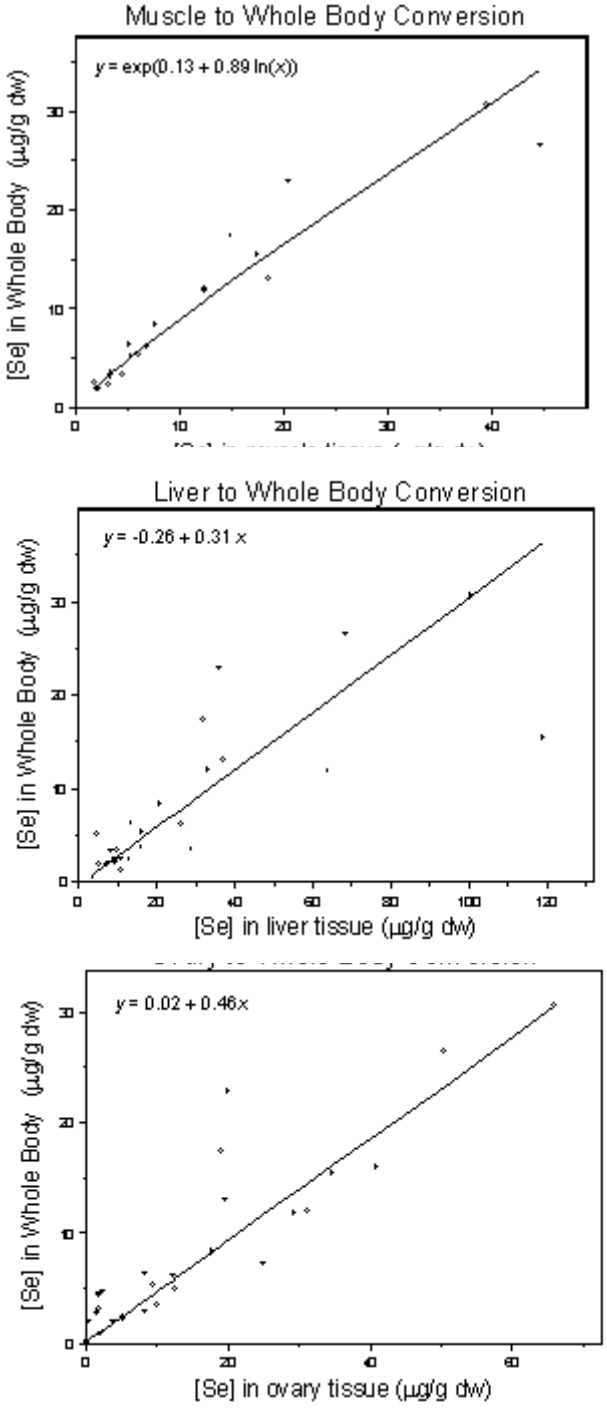


Figure 7. The quantile regression curves project median selenium concentrations in the whole body of bluegill, largemouth bass, tilapia and carp as a function of selenium concentrations in their tissues. Most data are from bluegill. Estimates of model parameters minimize the sum of weighted absolute deviations (see Appendix H for details about statistical analyses).

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. Pages 267-281 *in* B. N. Petrov and F. Csaki, editors. Second International Symposium on Information theory. Akademiai Kiado, Budapest.

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Koenker, R., and J. Gilbert Bassett. 1978. Regression Quantiles. Econometrica **46**:33-50.

Koenker, R., and J. A. F. Machado. 1999. Goodness of fit and related inference processes for quantile regression. Journal of the American Statistical Association **94**:1296-1310.

Koenker, R., and S. Portnoy. 1996. Quantile regression. Working Paper 97-0100, University of Illinois at Urbana-Champaign, College of Commerce and Business Admnistration, Office of Research, Urbana-Champaign.

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Comment:

Any new information or data that could potentially improve the quality of the document Literature Search

I performed a literature search for 2002 using the global search term “selenium” on relevant abstract databases. An additional reference that may have relevance to the proposed criteria is:

Assessment of exposure of larval razorback sucker to selenium in natural waters.
Beyers, D.W. and Sodergren, C. 2002. Archives of Environmental Contamination and Toxicology (NewYork); Vol.42 (1), pp. 53-59.

Response: So noted. We have obtained the following reports since completion of the draft document:

Beyers, D.W. and Sodergren, C. *Assessment of Exposure of Larval Razorback Sucker to Selenium in Natural Waters and Evaluation of Laboratory-Based Predictions.* Final Report to Recovery Implementation Program, Project CAP-6 SE. January 10, 2001.

Beyers, D.W. and Sodergren, C. *Evaluation of Interspecific Sensitivity to Selenium Exposure: Larval Razorback Sucker versus Flannelmouth Sucker..* Final Report to Recovery Implementation Program, Project CAP-6 SE-NF. January 10, 2001.

Comment:
Selenium in the Western Phosphate Resource Area - Background

I have described several studies underway related to selenium release in the Western Phosphate Resource Area. I add the following background to describe the context and importance of these studies. The Westem Phosphate Resource Area is responsible for 4% of the world's phosphate ore production and currently accounts for 15% of the domestic US production. It is regarded as a strategic national resource as it is the only source of elemental phosphorous in the nation. A one-hundred year ore supply has been documented and this resource will become increasingly important in the near future as phosphate in Florida becomes less available. Currently 5 companies are engaged in active mining in the WPRA and the phosphate industry accounts for over 70% of the non-farm income in the 3 S.E. Idaho counties.

In late 1996, selenium leaching from phosphate mining sites was observed following a case of equine selenosis in a down-gradient pasture (Möller and Talcott 1997). Since that time numerous studies have been conducted in the area to examine sources, pathways, receptors and controls of selenium in this unique lotic ecosystem. Selenium has been found to reside in the middle waste shales of the phosphoria formation in concentrations of up to 200 mg/kg or larger. Unlike selenium in the well studied areas of Belews Lake (flyash, powerplant discharge) and Kesterson National Wildlife Refuge (agricultural sump and drainage water), mobilized contamination from mining leachate at the WPRA can be regarded as monotonic in selenium[6]. Phosphoria, the calcium phosphate mineral mined in the WPRA, is actively used as a binding agent for heavy metals in HM contaminated site remediation. Other constituents of potential concern (i.e. Zn, Cd) have been identified in environmental surveys, however, they are of minor occurrence, geographically isolated and limited in relative risk. Sediment release from active and reclaimed mine sites into the Blackfoot River watershed has been an active environmental management concern.

Unique about the WPRA are the relative isolation of Se as a contamination vector in the affected watershed and the decades-long history of phosphate mining (and presumptive Se release) in the area. These attributes make the WPRA an exceptional field laboratory for examining Se dynamics and impacts to a watershed. With the levels of release observed,

[6] Se:S ratios in typical WPRA primary leachate are about 1:500.

phosphate mining in the WPRA is most certainly a target for the new criteria. Indeed a recent assessment identifies WPRA phosphate mining as the number one, human related factor in selenium environmental risk:

> *A selenium time bomb situation is developing in the United States and elsewhere that may result in substantial impacts on fish populations. The selenium time bomb has three components: (1) high food-chain bioaccumulation, (2) steep toxic response curve for fish, and (3) insidious mode of toxicity. If the threshold for selenium toxicity is exceeded, the time bomb explodes and a cascade of events is set into motion that will result in major ecosystem disruption. Several human-related factors are emerging that are capable of igniting the fuse of the time bomb by increasing waterborne concentrations of selenium and providing conditions favorable for bioaccumulation. Some of these factors are* ***(1) mobilization of selenium due to open-pit phosphate mining,*** *(2) use of constructed wetlands to treat selenium-laden wastewater from oil refineries and agricultural irrigation, (3) landfill disposal of seleniferous fly ash from coal-fired power plants, and (4) mobilization of selenium from animal feedlot wastes. Collectively, these threats may be sufficient to cause widespread, unanticipated toxic effects in fish populations. Only environmentally sound risk assessments followed by prudent management actions can defuse the selenium time bomb — once it explodes, it is too late to avoid significant impacts.*
>
> ***Selenium Impacts on Fish: An Insidious Time Bomb.*** A. D. Lemly
> Human and Ecological Risk Assessment: Vol. 5, No. 6, pp. 1139–1151 (1999)

The alarmist viewpoint expressed in the above paper has certainly not been borne out in 6 years of intensive, academic study of this mature site with substantial Se release and watershed deposition. Risk assessments based on water Se levels appear to overstate any negative biological effects, especially population level effects, in this lotic ecosystem. There have been no recorded observations of bird or fish population crashes in the area. Likewise there have been no observations, anecdotal or otherwise, of fish or bird terata in this actively fished and hunted area regarded for its trophy stock. Thus far in the examination of the area for biological impacts, two confirmed cases of livestock impacts have been observed. Direct selenium biological effects observations have been limited to one case of chronic equine selenosis (hoof wall dysplasia and alopecia) in confined animals with flood irrigated pasture and water from high concentration mine site runoff and one confirmed case of sheep deaths on seleniferous reclaimed mineland pasture following a late June snowfall. Livestock producers in this area, like most, supplement the mineral feed mix supplied to the animals with selenium, as is typical practice for animal health maintenance in the US.

The continuing large-scale wildlife effects field studies by University of Idaho researchers suggest the following:

- Lotic cold water ecosystems maintain selectively different selenium biodynamics that lentic warm water systems.
- Ecosystems with monotonic Se impacts are superior for isolating Se biodynamics in field studies than those with multiple stressor exposures such as agricultural pesticides in irrigation drainage.
- Current Se hazard assessment approaches and attitudes (vide supra) would have predicted wide-scale ecosystem collapse for the WPRA long ago. The lack of wildlife field observations of population level or organism level effects suggest the moderation of effects in this ecosystem may be due to: 1) site specific species, 2) lack of additional chemical or physical stressors found in more challenged environments such as power

plant cooling ponds and agricultural drainage sumps, 3) lentic and lotic system site specific differences, 4) the marginal Se status of the surrounding areas, or 5) weakness in the current approach to Se hazard assessment.

- Migratory populations of birds may experience a beneficial reproductive success effect from Se exposures at sites with significant selenium release.
- Birth defect rates for sampled cutthroat trout (1999, 2000) from the Se impacted Blackfoot River watershed are typical of background reproductive success statistics.
- For the Henry's Lake fish, no effects on fish growth, feed intake, or survival were found when fish were fed levels of dietary selenium, supplied as selenomethionine, as high as 10 mg Se/kg diet throughout the entire life cycle of the fish.
- Fish selenium depuration rates were highest for the highest dietary exposure groups and the residual whole body levels were lowest (Hardy, Figure 5).
- Reproductive success of the 2.5 year diet study fish is still in analysis. Preliminary visual analysis of the Henry's Lake treatment group data shows high variability of possible effects compared to controls, but this casual analysis does not reveal a dose-response relationship or a discernable pattern. Analysis of this recent data set (Hardy, Table 10) and the development of the Blackfoot River fish data set are incomplete at this time.
- Primary WPRA Se risk is at or in proximity to 1st order source release zones.

The results of these studies in the WPRA represent significant new knowledge in the management of environmental selenium..17

Response: So noted.

Comment: Challenges in Criteria Revision

I am aware of the challenges that EPA has in their work towards a revision of the aquatic biota criteria. I have been a quiet witness to the uncomfortable level of subjective passion that has characterized the scientific debate on Se ecosystem effects. As a practicing scientist working in related areas I am embarrassed by it all. I encourage passion for scientific discovery in my students and I drill them in disciplined objectivity about outcomes.

Science is first of all a set of attitudes. It is a disposition to deal with the facts rather than what someone has said about them...Science is a willingness to accept facts even when they are opposed to wishes... the opposite of wishful thinking is intellectual honesty. Scientists have simply found that being honest - with oneself as well as others - is essential to progress. Experiments do not always come out as one expects, but the facts must stand and the expectations fall. The subject matter, not the scientist knows best.

Skinner 1953, Science and Human Behavior.

Working though this passion is not easy for EPA in the preparation of the criteria. The draft document is respectful and science based. I teach my students: "regulatory science is science on

a deadline" and that decisions in the regulatory arena need to be made in a timely fashion. I am also aware that several years of large-scale Se focused ecosystem research in the WPRA is near completion. In fact since studies are active, it is a large resource of information to address current data needs. This year the University of Idaho has received a $900K grant from EPA to continue its work exploring the sources, pathways, receptors and control of selenium in the WPRA, in addition to the $2M of exploratory research thus far. This new knowledge will allow version 2.0 of the selenium criteria to be scientifically defendable and inclusive of a wider range of research observations.

Response: So noted. We recognize the problem.

In asking for information, data, and views from the public, on the topic of alternative criteria values, EPA emphasized that it was seeking only formal, fully transparent criteria derivation from primary data.

Comment:
Recommendations

1. Insert a sulfate adjustment for the acute freshwater criterion: *"For waters with >90% selenate as a fraction of total selenium, an adjusted selenium concentration of 185 + 0.19 [SO4 2-]* µg/L is protective of freshwater aquatic life."

2. Incorporate the Hardy data into the SMCV and GMCV calculations.

3. Recalculate the whole body-tissue selenium conversion using 3 parameter log normal regression. Use this data to recalculate SMCV and GMCV values.

4. Do not use a cold stress modifier to the FCV.

5. Strive to have an FCV that is not over- or under- protective of cold or warm water species. In the current analysis 9.5 mg/kg dw whole body Se approaches that goal.

6. Use a population level reference to broaden site specific application: *"The potential for reproductive failure in selenium exposed organisms makes population level protection important."*

Respectfully submitted,
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Response: Responses to the recommendations were addressed when first presented in this review. In summary, we agree with #1 and #2 and have revised accordingly. We also agree with item #6.

We handled the regression issue, #3, by a different route, quantile regression. Although we acknowledge the issues in #4, we retained the winter-stress downward adjustment in the 2004 draft. Regarding #5, the criterion concentration itself, we are expecting a mixture of responses to our request for scientific information, data, and views from the public. Our goal is to produce a criterion that is fully appropriate, neither under- nor over-protective across a wide spectrum of waters of the U.S.

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