EPA's Draft Tissue-Based Selenium Criterion:

A Technical Review

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Background

Selenium pollution of aquatic ecosystems is a significant global environmental safety issue. This is because selenium pollution is a common byproduct of several core economic activities including, but not limited to, irrigated agriculture, mining (coal, phosphate, uranium and numerous other sulfide minerals), coal-fired generation of electricity, and the refining of crude oil (1-6). Because selenium is often an unintended, but significant, component of commercial fertilizers (from the source rock used to make the fertilizer and/or from hazardous wastes, such as fly ash, legally disposed of in fertilizers) watersheds far removed from traditional sources of selenium pollution are also increasingly affected (7-9). Many aquatic ecosystems are sensitive to even low levels of selenium pollution and multiple toxic episodes have now been documented (10). Toxicity is typically expressed as impaired reproduction among populations of fish and/or aquatic-dependent birds (10). Due to these economic and environmental aspects, guidance for regulating selenium pollution is closely monitored by both the corporate-service scientific community (primarily, but not only, private-sector researchers and corporate-funded academia) and the public-service scientific community (primarily, but not only, government researchers and public-funded academia). Managers of commerce and managers of public-trust biotic resources (such as salmonids and waterfowl) both have vital interests that are directly influenced by the regulation of selenium pollution (11-13). The core regulatory guidelines for aquatic selenium pollution in the United States (U.S.) are the Aquatic Life Water Quality Criteria (Aquatic Life Criteria) derived by the U.S. Environmental Protection Agency (EPA) pursuant to the Clean Water Act (CWA) of 1977 (as amended). Because selenium is highly bioaccumulative and its

toxicity to fish and birds occurs primarily via dietary exposure, it is the long-term chronic criterion for selenium that is virtually always the controlling standard from a risk management perspective. EPA last promulgated an updated chronic criterion for selenium 17 years ago, in 1987 (14-15). EPA's current chronic criterion for selenium is 5 μg/L on an acid-soluble basis (16).

Controversy over the EPA chronic criterion emerges. During the past 17 years numerous researchers have estimated that the toxicity threshold for selenium lies below 5 μg/L (10, 17-23). In addition, three independently conducted studies funded by EPA since 1987 also reached the same conclusion (24-26). This body of work was produced predominantly by the public-service scientific community (27). More recently, a notable (11, 13) counter consensus predominantly from the corporate-service scientific community (27) has asserted that the current chronic criterion of 5 μg/L is overly restrictive (28-35). Critical reviews of the counter consensus focus on methodological deficiencies and the selective use of available literature and data (36-39). In another case (29), selective publication of their own analyses occurred after corporate-service authors were made aware that their full range of analyses provided strong support for toxicity guidelines endorsed by the public-service scientific community (40). Contributions from the corporate-service scientific community have sometimes been consistent with the public-service consensus regarding toxicity thresholds for lentic aquatic systems (28, 40), but not for lotic aquatic systems (28, 32-33). The core studies relied upon by the public-service scientific community are primarily from lentic systems (1, 3, 10, 21, 27, 39). Very recently, however, the first conclusive documentation of a toxic episode in a lotic system has been reported (41), and at modest levels of selenium pollution (6-32 μg/L waterborne selenium).

The paucity of lotic studies that match this recent study's (41) methodological rigor for detecting adverse effects suggests that our understanding of the vulnerability of lotic systems may be fairly uninformed, especially compared to the rich adverse effects databases from the much easier to study lentic systems (1, 10).

Even if lotic systems are less sensitive to selenium pollution; however, virtually all lotic systems serve either naturally (via floodplains) or artificially (via in-stream impoundments and off-stream water diversions) as source waters for lentic aquatic systems. From a risk management perspective, because of the hydrologic connections between lotic and lentic systems, it is the most sensitive system (lentic) that must dictate the controlling regulatory standards. A good illustration of this principle is provided by the hydrological system linking the Colorado River (lotic) to the Salton Sea (lentic) in southern California (10).

EPA prepares a draft updated chronic criterion. In 1997, EPA published a proposed set of Water Quality Criteria known as the California Toxics Rule, *aka* CTR (42). Pursuant to the Endangered Species Act (ESA) of 1973 (as amended), and prior to EPA promulgating the CTR, EPA was required to consult with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service (Services) and obtain the Services' concurrence that none of the proposed criteria in the CTR would jeopardize any ESA-listed species (43). Formal consultation between EPA and the Services was initiated in fall, 1997, and by spring, 1998, the Services had issued a draft "Jeopardy Opinion" based, in part, on the Services' evaluation that the 5 μg/L chronic criterion for selenium would likely jeopardize 15 ESA-listed species including species of fish, birds, amphibians, and reptiles. To avoid a final "Jeopardy Opinion" from the Services, EPA agreed to re-evaluate their CWA criteria guidance for selenium by 2002 (44). Re-evaluating the

selenium criteria guidance in the context of an ESA consultation raised new technical challenges for EPA.

EPA's normal procedure for setting Aquatic Life Criteria (45) does not directly consider toxicity data for aquatic-dependent wildlife (i.e., those species that depend on aquatic systems for food, but do not live and "breath" beneath the water's surface) and no separate Wildlife Criteria for selenium have been promulgated by EPA (13, 15, 46). Yet, the majority of the 15 ESA-listed species judged by the Services likely to be jeopardized by the current chronic criterion for selenium are aquatic-dependent wildlife (44). EPA's normal procedure is also much better suited for application to non-bioaccumulative pollutants, yet selenium is highly bioaccumulative (43, 46-47). Finally, for ESA-listed species, some of which are on the brink of extinction, both legally and biologically every individual of a population "counts" and therefore criteria guidance would need to be fully protective at an individual-effects level (43, 48).

EPA contracted with the Great Lakes Environmental Center (GLEC) to derive updated selenium criteria. To address the highly bioaccumulative nature of selenium, and concordant with expert consensus (15, 43, 47, 49), GLEC was instructed to derive the chronic criterion on a fish-tissue basis rather than on a water concentration basis. In March, 2002, EPA released the completed draft update document for selenium criteria (50). Largely, but not only, because the draft tissue-based chronic criterion was derived by GLEC employing an assumption that EC20 and LC20 levels of individual effects were acceptable, the draft chronic criterion of 7.9 μg/g, dry weight basis, was a nonstarter for ESA purposes (i.e., an LC20 level of allowable toxicity is far from fully protective). The U.S. Fish and Wildlife Service (FWS) immediately notified EPA of this and requested that EPA proceed no further with the draft criteria document (51).

The draft tissue-based criterion prematurely enters into decision-making arenas. For the past two years EPA has abided by the FWS request not to publish the draft criteria document in the Federal Register (13). However, during that period EPA also did not re-initiate the derivation of updated criteria on a basis that would be acceptable for ESA purposes and continued to make the draft criteria document available to the interested public. EPA has also created the appearance of supporting the draft document as sound science via public presentations before scientific professional societies (52-53) and via public statements (13). The draft tissue-based chronic criterion has been the subject of discourse in widely read scientific publications (12-13, 27), contributing to a developing perception within the regulated community of the draft guideline as quasi-officially sanctioned by EPA, i.e., but for a few bureaucratic formalities, the new chronic criterion for selenium. Consequently, EPA's draft criterion of 7.9 µg/g of whole-body fish tissue has prematurely made its way into environmental decision-making arenas and increasingly continues to do so. For example, West Virginia Senate Bill No. 353 was introduced January 30th, 2004, and seeks to replace West Virginia's current chronic criterion for selenium (5 µg/L) with the draft 7.9 µg/g tissue-based criterion effective September 1, 2004 (54). In Colorado, the draft tissue-based criterion has been introduced into the water standards regulatory arena (55). In California, water users within the federal Central Valley Project are citing the draft 7.9 µg/g tissue-based criterion as scientific support for seeking relaxed environmental terms and conditions on long-term water contract renewals that, once negotiated, would not be renewed again for at least 25 years (56-57). Decisions that may be irreversible for decades to come are being proposed based on the presumed scientific soundness of EPA's draft tissue-based chronic criterion for selenium.

Fundamental scientific flaws discovered in EPA's draft criterion proposal. Selenium standards and criteria recently emerged as a crucial issue among interest groups affected by the practice of mountain-top removal valley-fill coal mining (58-60). In this case, the difference between a 5 μg/L water criterion and a 7.9 μg/g tissue-based criterion is not trivial. One of us (JPS) was asked to conduct a detailed review of EPA's draft tissue-based criterion for selenium in response to questions emerging from the mountain-top mining controversy. As a result of that review and follow-up consultations with and amongst all co-authors of this paper, we discovered and confirmed several fundamental flaws that we believe are scientifically fatal for the draft criterion, not only for ESA purposes, but for any purpose. We discovered that the design implications of the controlling experiment from which EPA's draft 7.9 μg/g tissue-based criterion was derived had gone unrecognized by GLEC and EPA. We discovered that the crucial linear regression equation relating selenium concentrations in fish ovaries to concentrations on a whole-body basis was erroneously reported. We discovered that the assessments of risk to aquatic-dependent wildlife, if fish tissue were allowed to reach 7.9 μg/g selenium, were based on the 1995 draft of a wildlife toxicological benchmarks report rather than the much different 1996 final version. We discovered that the wildlife risk assessment was too narrowly focused on fish-eating birds. We discovered systematically incorrect wet-weight-to-dry-weight conversions of tissue concentrations for selenium. We discovered measures of selenium in aquatic invertebrates and fish liver tissue from a national database being erroneously plotted as data for selenium in whole-body fish tissue. In addition we discovered other less egregious errors. Most importantly, we found that all of the most egregious errors biased the final criterion recommendation in the same direction, toward dangerously overestimating the safely tolerable tissue-based number. Because this dangerously overestimated draft criterion has already taken on a quasi-official

status within scientific discourse (12-13, 52-53) and environmental decision-making arenas (54-57), we view as imperative the need for the fatal flaws we have discovered to be disseminated immediately and widely among scientists, natural resource managers, regulators, and policymakers. Therefore, we are submitting the following critical review for publication simultaneously with providing it to EPA.

Unrecognized Experimental Design of the Controlling Chronic Toxicity Study

GLEC's review of the scientific literature yielded 17 studies that were selected as the data pool from which an updated chronic criterion for selenium could be derived (50). GLEC followed EPA's standard procedures (45) as closely as possible and derived estimates of tissue-based chronic values for four genera of freshwater fish, including estimates of >11.64 μg/g for salmon and trout (*Oncorhynchus*), [<] 41.46 μg/g for fathead minnow (*Pimephales*), [<] 9.5 μg/g for bluegill sunfish (*Lepomis*), and < 17.50 μg/g for striped bass (*Morone*) (50) (where GLEC neglected to show a < sign that is, in fact, warranted, we have added it in brackets above). None of the genus chronic values could be estimated without substantial uncertainty (as indicated by the necessity of > and < signs). That outcome is a function of the available chronic toxicity data not being a very good fit for EPA's standard procedures (45).

A controlling chronic toxicity study is identified. However, GLEC noted that one of the 17 studies, Lemly's winter-stress study (20), was qualitatively distinct because in addition to a selenium treatment, the study included a simultaneous cold temperature stress similar to that faced in some degree by most natural fish populations during winter (winter stress). Because it was the only available study that incorporated the more realistic winter-stress design, and

because the study yielded an estimated chronic value lower than any of the uncertain genus chronic values noted above, GLEC quite reasonably chose to make Lemly's (20) experiment the controlling study for their criterion proposal. GLEC's draft tissue-based chronic criterion for selenium of 7.9 µg/g was adopted, unmodified, from the value Lemly reported for his selenium + winter stress treatment group, as measured at the end of the 180-day experiment (50). GLEC, following Lemly, associated that whole-body selenium concentration of 7.9 µg/g with 33.8 percent mortality of juvenile bluegill (50). GLEC did not clearly explain why there was no downward adjustment of the 7.9 µg/g concentration to bridge the gap between the attributed effects level of 30% mortality (on a control-adjusted basis) and the target effects level of 20% (EC20/LC20) that GLEC deemed appropriate for a criterion (50).

We support GLEC's decision to use the Lemly (20) winter-stress experiment as the controlling study for purposes of deriving a criterion. For more than 60 years it has been known that low winter temperatures substantively increase the toxicity of dietary selenium to birds (61-62), fish (20, 63-64), and mammals (65). Indeed, the selenium literature includes specific recommendations for considering and accounting for the effects of winter stress during hazard assessments (64).

Lemly's experimental design was more complex than GLEC recognized. Unfortunately, GLEC did not recognize the full complexity of Lemly's experimental design and its implications for estimating the magnitude of adverse effects. Lemly's study was a segmented time series experimental design that included periodic removal, without replacement, of surviving experimental fish (20). GLEC interpreted the study as if it were a much simpler experimental design, i.e., as if the selenium + winter stress treatments began with 210 fish (3 replicates of 70

fish each) which were all exposed to the treatment for 180 days, of which 71 died (71/210 = 33.8% mortality). GLEC may have been misled by the fact that Lemly reported only that same mortality quotient (20).

However, as clearly reported by Lemly, 30 of the 210 fish allocated to the selenium + winter stress treatments were removed before he initiated the experiment. The removed fish were used to establish baseline values for sublethal effects endpoints and tissue concentrations of selenium. Thirty additional surviving fish each were removed at days 60 and 120 of the experiment for intermediate measures of sublethal effects endpoints and tissue concentrations (20). Thus, unbiased direct measures of survivorship can only be derived within each distinct time segment of the experiment (i.e., days 1-60; days 61-120; days 120-180) because the number of fish entering each time segment was not the same as the number surviving the prior time segment. In other words, because 90 of the 139 fish that did not die during the experiment were exposed for less than the full 180 days of treatment (including 30 fish with zero exposure), the observed mortality count underestimated how many fish would have died had they all been exposed until they either died or survived the full 180-day treatment. A true effects estimate for the full 180-day treatment would account for the surviving fish that were removed periodically by the investigator and therefore were not available to suffer treatment-induced mortality. That can be accomplished by calculating the survival rates for each of the three time segments and then calculating the product of those three segment survival rates.

The true effects magnitude for the winter-stress selenium treatment was essentially 50% mortality. The relevant data are summarized in Table 1. For the selenium + winter stress treatment the time segment survival rates were 0.9167 (1-60 days), 0.6519 (61-120 days), and

0.8448 (121-180 days) respectively and the product of those three rates is 0.5048 (50.48% survivorship). Thus, the expected 180-day treatment mortality rate would be 49.52%. Similar calculations yield an expected 180-day control mortality rate of 4.19% (Table 1). Therefore on a control-adjusted basis, the effect level of Lemly's experiment was 47.31% mortality. Clearly, any tissue-based concentration associated with such a high level of mortality would constitute a fatally flawed criterion for protection of aquatic life and be scientifically inappropriate. Yet, because GLEC didn't recognize the complexities of Lemly's experimental design or the implications for assessing the true magnitude of toxicity, EPA has released a draft criterion that, at best (see next subsection), was essentially 50% lethal to juvenile bluegill fish.

The toxicologically controlling tissue value was probably 5.8 µg/g not 7.9 µg/g. It's likely that 7.9 µg/g is an overestimate of the tissue concentration necessary to cause the adverse effects observed in Lemly's study. Lemly (20) cautioned that the tissue concentration of 7.9 µg/g measured in fish from the selenium + winter stress treatment at day 180 was likely an artifact of severe lipid loss which reduced fish mass without reducing total selenium content of the fish (because lipids are essentially selenium-free; selenium is predominantly protein bound). Thus, the toxicologically controlling tissue concentration for risk assessment was the 5.8 µg/g reached by day 60 of exposure among fish in both the selenium + winter stress treatment and the selenium-only treatment. For fish in the selenium-only treatment, that is, in the absence of the severe lipid loss occurring after day 60 in the selenium + winter stress treatment, a whole-body selenium concentration of 5.8 µg/g was steadily maintained from day 60 to day 180. Therefore it was clearly established that 5.8 µg/g was the equilibrium tissue concentration to be expected

from consuming the 5 µg/g selenium feed used as the dietary exposure for both selenium treatments (20).

The clear implication from Lemly's discussion of his results is that a whole-body selenium concentration of 5.8 µg/g in juvenile bluegill as they enter the winter season (day 60 of the experiment) would be sufficient to cause 50% mortality and severe lipid depletion among fish still surviving by the end of winter (day 180 of the experiment). That severe lipid depletion in turn causes the selenium load in those surviving fish to become more concentrated. Accordingly, the terminal whole-body selenium concentration (7.9 µg/g) would be an artifact of toxic effects, triggered by the 5.8 µg/g of whole-body selenium the fish contained at day 60. In the absence of the 5.8 µg/g-triggered toxic effects (via lipid depletion), there would have been no increase in tissue selenium at day 180, as confirmed by the selenium only treatment. We agree with Lemly that this is the most parsimonious explanation of his experimental results and we expect that juvenile fish entering the winter season with 7.9 µg/g, as the current draft chronic criterion proposal allows, would result in even greater than 50% lethality.

Simple linear extrapolation [(7.9/5.8) x (47.3%) = 64.4%] yields an expectation of about 65% lethality. However, selenium toxicity response curves are distinctly nonlinear, and therefore linear extrapolation underestimates incremental increases in toxic effects to be expected from incremental increases in exposure (1, 4, 10, 41, 77). By comparison, for black-necked stilts (a species of shorebird) and the endpoint of selenium-induced embryo teratogenesis, the same proportional increase in exposure (1.36 times the 47.3% effects exposure concentration) causes the toxic response to increase from 47.3% to 90% (78). Consequently, we conclude that EPA's draft tissue-based chronic criterion for selenium of 7.9 µg/g would likely be associated

with the potential to cause on the order of 65-90% mortality of juvenile bluegill exposed to a winter stress challenge comparable to that simulated in the Lemly winter-stress study (20).

Correctly interpreted, EPA's controlling study indicates a tissue-based chronic criterion for selenium in the 4-6 µg/g range. Consequently, the controlling study for EPA's draft tissue-based chronic criterion, and the only study that incorporates a clearly demonstrated and environmentally widespread modifier of selenium toxicity (winter stress), is best interpreted as having demonstrated 50% lethality associated with a whole-body selenium concentration of 5.8 µg/g. The 50% lethality is not in question. Whether that effects level is judged by EPA to be associated with a tissue concentration of 5.8 or 7.9 µg/g is a matter of interpretation; however, either number would have to be substantially reduced to be an appropriately protective criterion, that is, to get the expected effects level down to the 0-10% level that is EPA's traditional goal for aquatic life water quality criteria (45, 50, 66). We believe that regardless of EPA's choice of interpretation, the appropriate criterion indicated by the Lemly winter-stress study (20) will likely need to be <5.8 µg/g on a whole-body fish tissue basis. For example, based on visual extrapolation from concentration-response curves available in the literature for whole-body fish tissue (50, 67-68), the ratio of the 50% effects whole-body concentration to the 10% effects whole-body concentration is roughly 1.75. Even 7.9 divided by 1.75 would yield a criterion estimate of 4.5 µg/g tissue selenium. Here it must also be considered that even 10% mortality may be unacceptable for ESA purposes. The public-service scientific community has identified 4-6 µg/g whole-body selenium in fish as the appropriately protective guidance for more than a decade (1, 4, 21, 39, 49).

Erroneous Presentation of a Crucial Regression Equation

The most sensitive endpoints for selenium toxicity in natural populations of fish and birds are measures of reproductive success. Therefore the preferred tissues for risk assessment are reproductive tissues such as eggs or ovaries (4, 15, 21-22, 47, 49-50), but reproductive tissues are available for sampling only seasonally and only at sites that support suitable breeding habitat. Consequently, whole-body tissue is a more practical measurement endpoint (15, 47, 49-50) making the relationship between selenium in whole-body tissues and reproductive tissues crucial for risk assessment (69). This is especially true for water bodies in moderate climates not subject to a strong winter-stress challenge. Where winter-stress is a strong challenge, the sensitivity of juvenile survivorship is comparable to more traditional reproductive endpoints (20). Clearly then, for a criterion based on a selenium concentration in whole-body tissue it is important to answer the question: "What will that whole-body chronic criterion translate to for eggs or ovaries?"

The erroneous regression equation presented in EPA's draft criterion document substantively misinforms risk assessment. GLEC developed a regression equation for translating between selenium concentrations in whole-body tissue and ovary tissue based on three sets of data (67, 70-71), although only two (67, 70) of the three sources for the data are identified in the applicable data appendix (50). A plot of the data is included in the draft criterion document and the regression equation of: [whole-body selenium] = 0.84 [ovary selenium] +0.45 is presented with the plot (Figure 4; 50). However, we observed that the plot showed the data pair (66 µg/g ovary selenium, 31 µg/g whole-body selenium) falling directly on the regression line. This would be possible only if either the regression equation had been

erroneously reported, or the data point had been plotted incorrectly. We re-calculated the regression equation using the same data listed in the data appendix and found that the correct regression equation for that data was: [whole-body selenium] = 0.45 [ovary selenium] + 1.32. For risk assessment purposes this difference is not trivial. Based on the erroneously reported regression equation, the proposed whole-body chronic criterion for selenium of 7.9 μg/g would translate to 8.9 μg/g in fish ovaries as opposed to an estimate of 14.8 μg/g from the correct regression equation. The former value would clearly be judged as safe and the safety of the later value would be a matter of interpretation. Alternative interpretations of the relevant literature have produced guidelines for reproductive toxicity thresholds ranging from 10-17 μg/g (22, 30). The public-service scientific community would consider 14.8 μg/g selenium in fish ovaries to exceed the threshold for reproductive toxicity among sensitive species.

Even the corrected regression equation is scientifically inappropriate. The corrected regression equation is valid only if the three data sets from which it was derived can be pooled together. Plotting each dataset separately we found that they yielded three clearly distinct regression relationships (Figure 1). There are straight forward reasons for the differences. The first dataset (70; Lemly 1982 in Figure 1) differed from the other two in that it is from a study that did not include a dietary exposure. Some authors suggest that the metabolic fate of selenium from water-only exposures is qualitatively different than that from exposures that include a dietary pathway (15, 43). With regard to the partitioning of selenium on a whole-body versus ovary basis that certainly appears to be true. Ovary selenium was always lower than whole-body selenium for Lemly's (70) water-only exposures. In clear contrast, ovary selenium was always higher than whole-body selenium for Coyle et al.'s study (67; Coyle et al. 1993 in Figure 1) that

included dietary exposures. GLEC had earlier reported in the draft criterion document that the scientific literature available for water-only exposures to selenium, and the associated whole-body toxicity thresholds reported in that literature, were excluded from consideration due to the lack of toxicological relevance of a water-only exposure pathway. We were therefore surprised to find water-only exposure data inappropriately pooled with data from dietary exposures for the purpose of calculating a regression equation relating whole-body selenium to ovary selenium. Clearly, the first dataset (70; Lemly 1982 in Figure 1) cannot be pooled with data from dietary exposures and must be excluded (just as all other water-only exposure data were excluded by GLEC).

Plotting the second dataset (71; Hermanutz et al. 1996 in Figure 1) required more effort. First, we did not believe it was appropriate to pool and average repeated measures of tissue selenium from within treatment groups (as done by GLEC) because doing so overestimates the strength of the regression (i.e., masks some of the variability in the raw data). Second, we used tissue-specific percent moistures reported specifically for bluegill (74% for whole-body tissue and 67% for ovary tissue; 72-75) to convert the Hermanutz et al. wet weight measures to a dry weight basis instead of the non-specific 80% "fish" percent moisture that GLEC applied to both types of bluegill tissue. The converted and plotted data revealed that although the Hermanutz et al. study included a dietary exposure pathway, it did not yield internally consistent results. Sometimes ovary selenium was higher than whole-body selenium (as would be expected for dietary exposure; 69) and sometimes it wasn't, thus the regression line falls mid-way between the internally consistent results of Lemly for water-only exposure and the opposite, but also internally consistent, results of Coyle et al. for exposure that includes a dietary pathway. We believe the mixed results follow from the Hermanutz et al. dataset representing a mix of data

from artificial streams that were being dosed with selenium on an ongoing basis and streams that were being allowed to recover (thus fish tissues were depurating) from prior dosing. Because portions of the Hermanutz et al. dataset are complicated by the differential depuration dynamics of whole-body versus ovary tissues, it also should not be pooled with the Coyle et al. dataset.

Appropriate translations of the proposed whole-body tissue-based chronic criterion to a reproductive tissue basis exceed all proposed toxicity thresholds. Of the three whole-body versus ovary datasets relied upon by GLEC, only the Coyle et al. dataset (67) represents an internally consistent equilibrium relationship between whole-body selenium and ovary selenium based on the predominant influence of dietary exposures as would be expected in nature. Based on the regression equation from the Coyle et al. dataset of: [whole body selenium] = 0.37 [ovary selenium] – 0.13, EPA's draft whole-body tissue-based chronic criterion of 7.9 μg/g would translate to 21.7 μg/g in ovary tissue. That estimate exceeds the entire range (10-17 μg/g) of alternative interpretations of the reproductive toxicity threshold for sensitive species of fish. For additional comparison, the most recent reproductive toxicity threshold rigorously documented in the published literature (for rainbow trout, based on field data) is 15.4 μg/g in eggs (41) [converted from 6 μg/g wet weight using the average percent moisture of 61.1% for rainbow trout and brown trout egg samples in the National Irrigation Water Quality Program's biota database (4, 76)]. Moreover, GLEC's data appendix includes a data pair from the Coyle et al. study (67) in which the whole-body selenium concentration (7.2 μg/g) in bluegill fish was very close to EPA's proposed draft tissue-based chronic criterion (7.9 μg/g). The ratio of ovary selenium to whole-body selenium for that data pair was 3.47 (50), a ratio very comparable to the factor of 3.3 recommended for generic hazard assessments (69). A ratio of 3.47 x 7.9 μg/g

translates to an ovary concentration of 27.4 µg/g. Employing the most scientifically appropriate translation factors, we estimate that a whole-body tissue-based chronic criterion for selenium of 7.9 µg/g would allow fish reproductive tissues to attain selenium concentrations (21.7-27.4 µg/g) exceeding even the most permissive toxicity threshold proposed to date (17 µg/g) by approximate 30-60% and to exceed the more cautious threshold (10 µg/g) recommended by the public-service scientific community by 117-174%. We believe that this outcome rises to the level of a second scientifically fatal flaw in EPA's draft chronic criterion proposal.

Inappropriate Basis for the Wildlife Risk Assessment

Although GLEC stated that their proposed draft chronic criterion was not developed with the intent of protecting wildlife, their draft criteria document contained a brief wildlife risk assessment. GLEC concluded from their risk assessment that the draft tissue-based criterion of 7.9 µg/g in fish would not cause unacceptable toxic effects for fish-eating birds (50). Aquatic life criteria are considered by EPA to be separate and distinct from wildlife criteria (43). Nonetheless, in the absence of promulgated wildlife criteria (as is the case for selenium), if the aquatic life criteria do not protect wildlife the purposes of the CWA are not being met (79). More critically, for waters of the United States supporting ESA-listed aquatic-dependent wildlife, the criteria would not be approvable for incorporation into state or tribal water quality standards (79). Thus, it would constitute more than just ecological folly to proceed with promulgation of an aquatic life criterion that demonstrably fails to protect aquatic-dependent wildlife.

GLEC's risk assessment was based on out of date information. The wildlife risk assessment presented in EPA's draft criteria document for selenium was based on information obtained from the 1995 revision of a U.S. Department of Energy report, *Toxicological Benchmarks for Wildlife* (80), and neglected the 1996 final revision of the same report (23). We refer to these two reports as Benchmarks 95 and Benchmarks 96. All of the information relied on by GLEC from Benchmarks 95 was updated in Benchmarks 96 and the updated information substantively alters the risk assessment outcomes and the conclusions that can be drawn from those outcomes. Here we focus on the risk assessment information in the Benchmarks reports that is based on toxicity data for selenomethionine because that is the form of selenium used in laboratory toxicity tests that is most relevant to avian dietary selenium exposures in nature (81).

Employing bioenergetic equations and allometric scaling between laboratory test species and risk assessment species the Benchmarks reports presented estimates for dietary NOAEL's and LOAEL's on a wet weight basis. GLEC focused on the Benchmarks 95 results for three fish-eating bird species. GLEC first converted the dietary NOAEL's and LOAEL's to a dry weight basis assuming 80% moisture for a fish diet. Then GLEC calculated the geometric mean of the NOAEL and LOAEL for each species which they equated to a maximum acceptable dietary toxicant concentration (MATC) for each species. Finally, the MATC's were compared to the draft fish tissue-based chronic criterion for selenium of 7.9 μg/g (50).

The three dietary MATC's reported by GLEC ranged from 10.61 to 12.20 μg/g (Table 2, first column). Because all of those estimates of the maximum acceptable dietary exposures to selenium exceeded 7.9 μg/g, GLEC concluded that the draft tissue based chronic criterion would protect wildlife (50). Using the same methods GLEC used, but employing the revised and more up to date information from Benchmarks 96 for the original three assessment species and an

additional species of aquatic-dependent bird included in Benchmarks 96, but not included in Benchmarks 95, we calculated a range for dietary MATC's of 3.73 to 20.31 μg/g (Table 2, second column). Two of our four estimated MATC's are lower than 7.9 μg/g. Finally, we calculated MATC's from Benchmarks 96 using a more realistic estimate of 75% moisture for a fish diet. A moisture content for whole-body fish tissue of 75% is the value commonly cited in selenium literature (22, 27, 41) and for 57 species of freshwater fish in the National Irrigation Water Quality Program biota database the median percent moisture was 74.5% [only 4 species averaged as high as 80% moisture (4, 76)]. The difference between using 75% moisture or 80% moisture is the difference between multiplying wet weight values by a factor of 4 or a factor of 5 to convert them to dry weight values. Thus, GLEC's use of 80% moisture introduced a systematic 25% bias in the direction of overestimating MATC's. Our final set of MATC's were 4.46 μg/g for belted kingfisher, 12.88 for great blue heron, 16.25 for osprey, and 3.34 for American woodcock (Table 2, third column). Our estimated MATC's for the American woodcock were calculated assuming a diet comprised predominantly of earthworms and therefore were based on the typical percent moisture of earthworms, not the percent moisture of fish (Table 2). Based on these four assessment species, the draft tissue-based chronic criterion for selenium of 7.9 μg/g would leave a substantive proportion of aquatic-dependent wildlife species unprotected; perhaps on the order of half the species.

The narrow focus on fish-eating birds as the assessment species neglects the more rigorous basis for wildlife risk assessment offered by other species. One of the weaknesses of relying on the Benchmarks reports for wildlife risk assessment is that there are numerous assumptions and uncertainties involved. The realism of the estimated MATC's is very difficult

to evaluate. Once it is realized that proposing to allow fish tissue to reach 7.9 µg/g selenium has implications for the rest of the aquatic food chain, wildlife risk assessment doesn't have to be confined to assessments based on fish-eating birds. That allows the risk assessment to move away from modeled (virtual) outcomes and toward empirical (real) outcomes documented for such species as the mallard duck. Additionally, fish-eating species of birds have not been found to be as sensitive to selenium as various species of ducks and shorebirds whose breeding-season diet is comprised primarily aquatic invertebrates (82-83).

It has been rigorously estimated for the mallard duck, based on multiple experimental feeding studies, that the dietary EC10 for selenium-induced reproductive effects is 4.87 µg/g with a 95% confidence interval of 3.56-5.74 µg/g (77). For the sake of providing the effects measure that GLEC would have used, the estimated EC20 is 5.86 µg/g (95% C.I. = 4.68-6.64 µg/g), but as previously noted a 20% effects level would not produce criteria estimates that meet ESA purposes. The estimated EC01, a more ESA-compatible reference point, is 2.82 µg/g (95% C.I. = 1.56-3.78 µg/g). To put these rigorous effects data for mallards to use, an estimate of how much selenium aquatic invertebrates would contain in environments sufficiently polluted to produce fish with 7.9 µg/g whole-body selenium is required?

The most rigorous experimental study of the relationship between aquatic invertebrate selenium and fish whole-body tissue selenium, which utilized radio-labeled selenium, concluded that the invertebrate-to-fish concentration factor was 0.5 across a range of foodborne (invertebrate) selenium concentrations (84). Other experimental studies have produced similar results (85-89). At a concentration factor of 0.5 the invertebrate food chain would have to contain about 15.8 µg/g selenium (i.e., 7.9/0.5) to produce fish with 7.9 µg/g. That would be equivalent to the dietary EC95 for reproductive toxicity to mallards (77). In other words,

allowing fish tissue to reach 7.9 μg/g would allow a level of contamination in the other parts of the aquatic ecosystem sufficient to cause nearly total reproductive failure among mallard ducks. As is the case for all lab studies, the realism of these lab-to-field extrapolations is fraught with uncertainty (10, 84, 90).

As a check on the realism of lab-generated invertebrate-to-fish concentration factors, comparison to field data is desirable. For this purpose we queried the biota database of the National Irrigation Water Quality Program (4, 76) and summarized the spatially and temporally matched samples of fish and aquatic invertebrates from sampling sites where whole-body fish tissue averaged between 5 and 10 μg/g selenium (a concentration range focused on the data that falls near the draft tissue-based criterion of 7.9 μg/g). The implied invertebrate-to-fish concentration factors from this dataset ranged from 0.67 to 1.36 (Table 3). These results suggest that the selenium content of aquatic invertebrates in ecosystems sufficiently contaminated to produce fish with 7.9 μg/g would fall in the range of 5.8-11.8 μg/g. Such a range of dietary exposure for mallards would correspond with an EC20 to EC85 range of toxic effects based on reproductive toxicity (77). The results of our database query also suggested a central tendency for the implied concentration factors of about 1.1 (Table 3). Thus, for wildlife risk assessment purposes, 7.9 μg/g in whole-body fish tissue might most reliably be considered to translate to about 7.2 μg/g in aquatic invertebrates. This estimate exceeds even the upper 95% statistical confidence boundary (6.64 μg/g) of the dietary EC20 for mallards and equals about the EC40 (77). In summary, allowing fish whole-body tissue to contain as much as 7.9 μg/g selenium would allow levels of aquatic food chain contamination highly likely (>95% statistical confidence) to exceed the dietary EC20 for reproductive toxicity in mallards, with a best-estimate likelihood of an EC40 level of adverse effects and the outside possibility of EC85-95

levels of adverse effects. We conclude that this clear lack of protection for aquatic-dependent wildlife provided by EPA's draft chronic criterion once again rises to the level of a scientifically fatal flaw.

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TABLE 1. EXPERIMENTAL RESULTS FROM LEMLY WINTER-STRESS STUDY (20).

	Cold-Control	Cold-Selenium
Fish Allocated to Treatment	70	210
Fish Removed for Baseline Samples Before Treatment	10	30
Fish Removed as Intermediate Samples:		
Day 60	10	30
Day 120	10	30
Day 180	10	30
Raw Number of Fish Deaths:		
Days 1-180	2	71
Fish Treated:		
Days 1-60	60	180
Days 61-120	49	135
Days 121-180	39	58
Fish Surviving:		
Days 1-60	59	165
Days 61-120	49	88
Days 121-180	38	49
Segment Survival Rates:		
Days 1-60	0.9833	0.9167
Days 61-120	1.0000	0.6519
Days 121-180	0.9744	0.8448
Days 1-180	**0.9581**	**0.5048**
Full Treatment Mortality Rates	**4.19%**	**49.52%**
Full Treatment Control-Adjusted Mortality Rates	**N/A**	**47.31%**

TABLE 2. COMPARISON OF WILDLIFE RISK ASSESSMENT OUTCOMES BASED ON DIFFERENT SOURCES AND METHODS
(Maximum Acceptable Toxicant Concentrations, MATC's, based on toxicity data for dietary exposure to selenomethionine)

Wildlife Species	MATC 1995 Benchmarks 80% Moisture (fish)	MATC 1996 Benchmarks 80% Moisture (fish)	MATC 1996 Benchmarks 75% Moisture (fish)
belted kingfisher	10.61 μg/g, dw	5.58 μg/g, dw	4.46 μg/g, dw
great blue heron	12.02	16.09	12.88
osprey	12.2	20.31	16.25
American woodcock	No Data	3.73	3.34

Note: MATC for American Woodcock in the last column is based on 77.7% moisture in worms. The estimate of percent moisture in earthworms is based on United States Fish and Wildlife Service file data; n = 83

TABLE 3. MATCHED SAMPLES OF FISH AND AQUATIC INVERTEBRATES FROM SAMPLING SITES WHERE THE FISH SAMPLES AVERAGED 5-10 μg/g SELENIUM, DRY WEIGHT

Location	Invertebrate Selenium	Fish Selenium	Implied Concentration Factor
Colorado	4.8 μg/g	5.3 μg/g	1.10
Utah	4.4	6.0	1.36
Utah	4.4	5.2	1.18
Utah	8.2	10	1.22
Utah	8.4	9.4	1.12
Utah	7.6	5.7	0.75
Utah	6.9	6.7	0.97
Montana	4.8	6.1	1.32
Montana	9.2	5.3	0.67
Median Concentration Factor			1.12
Average Concentration Factor			1.08

Source: National Irrigation Water Quality Program biota database (4, 76)

Figure 1. Regression lines for the three whole-body versus ovary datasets in Appendix G of EPA's Draft Criteria Document for Selenium (50). All three lines are statistically significantly different from each other ($p<0.05$). Lemly 1982, Hermanutz et al. 1996, and Coyle et al. 1993 are references 70, 71, and 67 respectively. The regression equation for Lemly 1982 is: $Y = 2.02X - 0.0325$; $R^2 = 0.970$. The regression equation for Hermanutz et al. 1996 is: $Y = 0.604X + 1.24$; $R^2 = 0.815$. The regression equation for Coyle et al. 1993 is: $Y = 0.369X - 0.126$; $R^2 = 0.970$. The Hermanutz data pairs were plotted individually (instead of pooling and averaging replicates as was done by GLEC) and were converted from wet weight to dry weight values using tissue specific percent moisture values for bluegill fish (74% for whole-body tissue; 67% for ovary tissue). (ppm = µg/g, dry weight)