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ASSESSMENT OF SELENIUM FOOD CHAIN TRANSFER AND CRITICAL EXPOSURE FACTORS FOR AVIAN WILDLIFE SPECIES: NEED FOR SITE-SPECIFIC DATA

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ABSTRACT: Observations of selenium poisoning in Belews Lake, NC in the mid-1970s and Kesterson Reservoir, CA in the mid-1980s precipitated a large number of selenium studies. Numerous authors have evaluated the potential for selenium to cause ecologically significant effects via food chain transfer in aquatic ecosystems, especially wetlands. Additionally, bioaccumulation models have been proposed for estimating selenium concentrations in food chains and water that should not be exceeded in order to avoid reproductive effects in avian and aquatic species. The current national chronic ambient water quality criterion (WQC) for protection of aquatic life is 5 μg/L. Scientists with the U.S. Fish and Wildlife Service have recommended setting the ambient water quality criterion at 2 μg/L for both aquatic and wildlife protection.

Reported site-specific variations in selenium's effects on aquatic life and birds prompted us to re-evaluate the basis for the 2 μg/L recommendation, and in particular one of the wildlife bioaccumulation models used to support this value. We used a

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probabilistic approach to assess water, food chain, and bird egg residues from 15 sites. Our data evaluation indicates significant differences in selenium accumulation in invertebrates and bird eggs among sites and among species. Both a two-step regression model (water → food chain → bird eggs) and a one-step regression going directly from waterborne selenium (WS) to mean egg selenium (MES) were fitted to all data for 15 sites and four bird species. The one-step model contained less variability than the two-step model and had a coefficient of variation (r^2) of 0.67. Uncertainty analysis of the regression models provided a distribution of waterborne selenium concentrations associated with bird egg tissue residues. Using the 10th and 50th percentiles of these distributions, we calculated waterborne selenium concentrations between 6.8 and 46 µg/L that are protective of birds. These values are associated with an effects threshold of 20 mg/kg selenium dry weight in bird eggs, which is the EC_{10} for mallard duck embryo teratogenis (Skorupa et al. 1996). The 10th percentile of this distribution, 6.8 µg/L, is slightly above the EPA water quality criterion of 5 µg/L. The water concentrations protective of birds range from slightly more than the current EPA WQC (6.8 vs. 5.0 µg/L) to a factor of 10 or greater at some sites.

Our results also indicate a reasonably strong correlation between water and mean egg selenium concentrations. However, site-specific factors strongly influence this relationship, and when waterborne selenium approaches or exceeds the WQC, collection of site-specific data would be appropriate to accurately assess the WS to MES relationship. Evaluation of the site-specific relationship between WS and MES can determine whether site-specific differences are important and whether or not the EPA WQC is likely to be over protective of bird populations. In this paper, we question the need for the WQC to be set at 2 µg/L to protect aquatic birds. Overall, bird-egg residues appear to be the best tool for assessing potential for risk to birds from selenium.

KEYWORDS: selenium, birds, bioaccumulation, food-chain, invertebrates, eggs, water quality criteria

Selenium is a metalloid element which, for a long time, was of interest due to its potential for causing livestock toxicity and its necessity as a nutritional supplement in livestock feed in certain areas of the United States (Rosenfeld and Beath 1946, 1964). Inclusion of selenium in the list of 65 priority pollutants (subsequently expanded to 129) in the mid-1970s provided greater focus to developing aquatic toxicology data for derivation of a selenium water quality criterion (Adams 1976, Cardwell et al. 1976, U.S. EPA 1979, Halter et al. 1980, Adams and Johnson 1981).

With continued toxicity testing and field studies, the waterborne selenium concentration thought to be protective of aquatic life has decreased. Adams and Johnson (1981) suggested 52 µg/L might be adequate for freshwater species; the first EPA water quality criterion (U.S. EPA 1980) recommended acute values (criterion maximum concentrations) of 54 and 35 µg/L, respectively for saltwater and freshwater organisms with no recommendation for a chronic criterion; and the most recent EPA water quality criterion has been set at 20 and 5 μg/L to protect against acute and chronic reproductive effects, respectively, in freshwater fish and invertebrates (U.S. EPA 1987). The U.S. Fish and Wildlife Service (USFWS) has supported reducing the freshwater water quality criterion to 2 μg/L to protect both aquatic life and birds (Lemly 1993, 1996, Lemly and Smith 1987, Skorupa et al. 1996). As additional studies were performed, it became apparent that it was important to consider dietary exposure when assessing selenium's toxicity to both aquatic organisms and birds. Numerous studies have demonstrated selenium's incorporation in the eggs of both birds and fish can result in reduced egg hatchability, offspring deformities, and increased juvenile mortality (Bennett et al. 1986, Heinz et al. 1989, Hoffman and Heinz 1988, Woock et al. 1987).

Two events in the late 1970s and early 1980s dramatically influenced the extent and nature of selenium research. The first event, reported by Cumbie and Van Horn (1978), assessed selenium effects on fish populations in Belews Lake, North Carolina, over a two-year period. This lake was receiving fly ash from a coal-burning electric power facility which increased lake selenium concentrations. Cumbie and Van Horn observed effects on fish populations at selenium concentrations as low as 10 μg/L, whereas populations in one portion of the lake appeared unaffected at < 5 μg/L (U.S. EPA 1987). The latter value has since been used by U.S. EPA as the chronic water quality criterion for selenium. Data subsequently released indicate the actual selenium concentration was 4 μg/L (Sorenson et al. 1984). Other researchers (Finely 1985, Woock et al. 1987), using bluegills (*Lepomis macrochirus*) from the same lake, subsequently implicated dietary transfer of selenium as critical to understanding and predicting its toxicity to aquatic species. More recently, laboratory studies involving both water and dietary exposures have confirmed Woock's findings concerning maternal transfer of selenium (Coyle et al. 1993, Schultz and Hermanutz 1990).

The second event occurred in the mid-1980s when selenium in agricultural irrigation drain water was implicated in extensive poisoning of fish and birds at Kesterson National Wildlife Refuge, California, (Saiki and Lowe 1987, Ohlendorf et al. 1986, 1988). It is now recognized that weathering of cretaceous marine shales may produce soils with elevated selenium levels. Such soils occur in many parts of the western U.S., including areas in California, Nevada, Wyoming, Utah, and Colorado (Presser 1994). Many of these areas have low rainfall and crops are grown by irrigating the soils. Irrigation can leach selenium from these soils, increasing concentrations in irrigation drain water, some of which is returned to streams, impoundments and lakes. This same leaching process is the reason many lakes and some streams and rivers in the western U.S. contain elevated levels of selenium derived from natural deposits both in water and sediments (Birkner 1978, Chau et al. 1976, Adams and Johnson 1981).

Numerous studies following those at Kesterson have documented selenium's ecotoxic potential. As a result of these studies, various bioaccumulation models have been proposed for estimating concentrations of water, sediment, and dietary selenium that should not be exceeded in order to avoid reproductive effects in birds and aquatic life (DuBowy 1989, Skorupa and Ohlendorf 1991, Peterson and Nebeker 1992, Van Derveer and Canton 1997).

Recently, field studies were performed in the wetlands on Kennecott Utah Copper property along the south shore of the Great Salt Lake (ep&t and Parametrix 1997). These wetlands contain many of the same species reported to be affected by selenium at Kesterson Wildlife Refuge: American avocets (*Americana recurvirostra*), black-necked stilts (*Himantopus mexicanus*), coots (*Fulica americana*) and several other waterfowl and shorebirds. Selenium concentrations in avocet and stilt diets exceeded levels reported by Lemly (1995) and Skorupa et al. (1996) as associated with reproductive effects. Field studies failed to demonstrate differences (in reproductive success) between birds using Kennecott properties (N = 55 to 112 nests, depending upon species) and birds using two reference sites bordering the Great Salt Lake (N = 59 to 144 nests).

Other areas in the U.S. (South Dakota, Colorado, and Ohio) have also reported elevated selenium levels without apparent effects on aquatic life. Data from these areas have been used to derive site-specific water quality criteria for streams that are two to five times higher than the national ambient water quality criterion for selenium (Reash et al. 1988, Times Limited et al. 1996, Canton and Van Derveer 1997, Van Derveer and Canton 1997). Evidence of site-specific differences has prompted us to re-evaluate some of the food chain bioaccumulation models used to derive water concentrations protecting aquatic life and wildlife resources, particularly those proposed by Skorupa and Ohlendorf (1991) and Ohlendorf and Santolo (1994). We use this re-analysis to demonstrate the importance of site-specific data in setting a selenium water quality criterion.

OVERVIEW OF SKORUPA AND OHLENDORF (1991)

Skorupa and Ohlendorf (1991) constructed a model to focus on an upper bound estimate of avian bioaccumulation potential. They postulated that their model represented bioaccumulation of selenium in eared grebes and confirmed that it closely predicted the mean egg selenium (MES) measured in eared grebes on four evaporation ponds in the San Joaquin Valley, California. They also developed confidence intervals for this eared grebe model and used their results to estimate a general avian threshold.

The Skorupa and Ohlendorf model is comprised of two separate linear regression models that are combined algebraically. The model assumes selenium moves from water into the aquatic food chain, then is consumed by birds and transferred maternally to bird eggs (Lemly and Smith 1987). The movement of selenium in this food chain can be expressed as water selenium (WS) → food chain selenium (FCS) → dietary selenium (DS) → mean egg selenium (MES). The WS → FCS relationship was based on field data collected from several sites within the San Joaquin Valley, California, and the DS → MES relationship was based on experimental studies with game-farm mallards (*Anas platyrhynchos*) exposed to a selenomethionine-spiked diet (Heinz et al. 1989).

The first model component is a regression involving WS → FCS. Skorupa and Ohlendorf evaluated several regressions developed by Shelton et al. (unpublished) for this model component. Specifically, they evaluated regressions developed using Tulare Basin field data for four invertebrate taxa: corixids, chironomids, damselflies, and brine shrimp.

Each regression was combined algebraically with the one developed by Heinz et al. (1989) and evaluated for best fit with field data from the San Joaquin Valley, as discussed below.

In considering Skorupa and Ohlendorf's model evaluation, it is important to understand their species selection for MES. They based MES on values measured in eared grebes (*Poacips nigricollis*). Skorupa and Ohlendorf used eared grebes as an example of maximum bioaccumulation potential in birds, based on such factors as home feeding range, residency time, and diet as compared with other species (e.g., mallards, avocets, stilts). Their analysis revealed the brine shrimp regression best fit the eared grebe data even though they apparently did not feed on brine shrimp at the sites evaluated.

Using this approach, Skorupa and Ohlendorf's two-step model was comprised of WS → FCS (brine shrimp) and DS → MES (game-fed mallard), with FCS and DS assumed equal for the eared grebe. The condition FCS = DS was postulated to hold for the eared grebe because of its small home range, long residency time, and dietary considerations. This model was used to estimate the WS concentration that would result in a specified MES concentration. Skorupa and Ohlendorf examined selenium-egg data for 74 sample means collected from areas believed to contain natural background levels of selenium and estimated the upper 90th percentile MES to be 2.9 mg/kg. This value was rounded to 3.0 mg/kg by the authors and was termed the "contamination threshold."

The MES concentration of 3.0 mg/kg was entered into the model to derive a point estimate of the WS concentration (0.5 μg/L) that would produce the contamination threshold in bird eggs. Using a graphical approach that matched the lower 95% confidence bands for each model component, Skorupa and Ohlendorf estimated 2.3 μg/L as the WS that would not result in exceedance of the MES contamination threshold (Figure 1). Their estimate corresponds closely with the value (2 μg/L) that has been recommended as a chronic selenium water quality criterion by the USFWS (Lemly 1993, 1995, 1996) for wildlife protection.

Ohlendorf and Santolo (1994) used this same model with an effects threshold of 8 mg/kg MES to predict a WS effects threshold of $\leq$ 7.8 μg/L (Figure 1). The 8 mg/kg MES value was based on the threshold for impaired egg hatchability in bird populations in the Tulare Basin (Skorupa and Ohlendorf 1991).

METHODS

We have applied the log-linear model form used by Skorupa and Ohlendorf to a more encompassing data set that includes data from many sites, food-chain species, and bird species in the western United States. We refer to this model as a "global" model because it estimates (1) the mean relationship over many sites and species and (2) the overall uncertainty and variability in applying a single model to different sites and species.

Like the Skorupa and Ohlendorf model, our model has two components that reflect different empirical regression relationships (WS → DS and DS → MES). We also developed a model based on a one step regression (WS → MES). Following Skorupa and Ohlendorf, we distinguish between selenium concentrations in individual food chain species (FCS) and dietary selenium (DS), which for our model is estimated by applying

Figure 1. Skorupa and Ohlendorf (1991) , Ohlendorf and Santolo (1994)

food fractions and limited spatial averaging to represent exposure over an area. We calculated an estimated DS term, which is to be distinguished from true DS, a value that would be known in a laboratory experiment but is not feasible to measure in a field study. We then evaluated potential effects of species and site variability on each of the regression relationships (WS → FCS, WS → DS, DS → MES, and WS → MES).

Finally, we applied probabilistic techniques to quantify the uncertainty associated with using this model and derive a distribution of potential selenium water quality criteria for each model, rather than a single criterion. Our approach considers the variation observed across sites in the empirical data, whereas a single criterion provides a conservative value protective of most sites, but also overprotective of many sites. The variability we describe has implications from a risk management perspective and a site-specific water quality criterion perspective for NPDES permits.

Data Set Selection and Preparation

The database we used was based on selenium tissue (invertebrate, plant, and egg) and water concentrations from USFWS and USGS reports for 15 western sites (Table 1). The sites covered a broad range of physical and ecological conditions. This heterogeneity enhances the representativeness of the global model and also appropriately encompasses all the uncertainty in applying such a model to any given site and species.

Data for each site included different areas (e.g., Kesterson Reservoir within San Joaquin Valley), and different ponds (e.g., Pond #2 at Kesterson Reservoir) within an area. All waterborne selenium concentrations were converted to µg/L. All tissue concentrations were reported as mg/kg dry weight in the data sources and were maintained as such in this paper.

The data were preprocessed to provide paired data ({WS, FCS}; {FCS, MES}; {WS, MES}) suitable for regression. Mean WS, FCS, and MES values provided in the reports were assumed co-located if they were reported for the same pond and study period. Where mean water and food chain selenium were reported for a collection of ponds sampled during the same period, data were also assumed to be co-located. Where several tissue concentration means were reported for a given pond and taxon and for the same study period, they were averaged. If more than one WS and FCS value were reported for a given pond (or collection of ponds) and study period, the values were averaged to provide a better estimate of the spatially averaged exposure concentration. The study focused on the avocet and stilt, both of which have relatively small home ranges. Therefore, averages were not calculated for large areas involving many ponds. It is recognized that the limited spatial averaging might systematically overestimate exposure concentrations for the mallards and coots which have larger exposure ranges.

TABLE 1--Studies used as data sources.

Sites	References
American Falls Reservoir Area - Idaho	Low and Mullins 1990
Belle Fourche Reclamation Project - South Dakota	Roddy et al. 1991
Benton Lake NWR[1] - Montana	Nimick et al. 1996
Bosque Del Apache NWR - New Mexico	Ong et al. 1991
Bowdoin NWR/Adjacent Areas of the Milk River Basin - Montana	Lambing et al. 1988
Middle Green River Basin - Utah	Stephens et al. 1988, 1992
Kendrick Reclamation Project Area - Wyoming	See et al. 1992
San Joaquin Valley - California	Moore et al. 1989, 1990
Klamath Basin - California/ Oregon	Sorenson and Schwarzbach 1991
Malheur NWR - Oregon	Rinella and Schuler 1992
Pine River Project Area - Colorado/ New Mexico	Butler et al. 1993
Riverton Reclamation Project - Wyoming	Peterson et al. 1991
Sacramento NWR Complex - California	Dileanis et al. 1992
Sun River Irrigation Project - Montana	Nimick et al. 1996
Sweitzer Lake - Colorado	Butler et al. 1991

[1] NWR = National Wildlife Refuge

Dietary selenium for each bird species was calculated for each pond as a weighted average of the invertebrate and plant taxon means, with the weight representing dietary fractions reported in the literature for each bird species (Table 2). The actual dietary fractions used at a given pond varied somewhat from the fixed values in Table 2 when a particular taxon was not present in the data set. If a component of the dietary fraction for a taxon was not present, then dietary selenium was adjusted so the remaining dietary fractions totaled 1.0 (by dividing the sum of the weighted components that were present by the sum of their weights).

Limitations of the Data Set

Our data set reflects a number of empirical inconsistencies due to combining different studies. For example, different sample sizes contributed to means for each pond (e.g., Pond #2) within an area (e.g., Kesterson Reservoir) or site (e.g., San Joaquin Valley), and different food items and avian species were measured. In addition, potentially different sampling, compositing, and analytical methods were applied. Finally, the food chain data set was weighted toward corixids and chironomids for dietary selenium because these were the predominant species sampled. However, the data sources indicate that the macroinvertebrates most abundant at a site were sampled. Since most of these bird species are considered opportunistic feeders, the most abundant macroinvertebrates would presumably dominate bird diets at a given site.

TABLE 2--Dietary feeding percentages.[1]

Taxon	Black-necked Stilt	American Avocet	American Coot	Mallard[3]
Hemiptera	35	...	...	...
Coleoptera	32	11	5	...
Diptera	10	24	...	10
Odonata	7	...	5	...
Mollusca	8	...	...	...
Crustacea	1	9	...	...
Miscellaneous[4]	6	21	6	57[5]
Vegetation	1	35[2]	84	33

[1] Feeding percentages based on Bogiatto 1990, Cogswell 1977, Elridge 1990, Jones 1940, Martin et al. 1951, Ryser 1985, Wetmore 1925.

[2] Although specific percentages are not given, Hothem and Ohlendorf (1989) report a much lower vegetation feeding fraction for avocets and an overall diet more similar to stilts than is presented in this table.

[3] Estimated mallard diet during breeding season.

[4] Miscellaneous refers to unidentifiable food prey items for all species except mallards (see footnote 5).

[5] Miscellaneous for mallards consists of unspecified proportions of annelids, snails, caddisflies, ostracods, odonates, and coleoptera.

Model Estimation

Our modeling effort began by using a series of least squares regression equations to investigate differences in bioaccumulation at different sites and for different species. It also focused on understanding the uncertainty in developing a single empirical model for selenium bioaccumulation in birds. By using data from all the studies listed in Table 1, a global regression model was developed for each component of the avian food web model, namely waterborne selenium (WS) to food chain selenium (FCS) and dietary selenium (DS) to mean egg selenium (MES). Models also were developed for specific sites and species for which data from four or more distinct ponds were available. Comparisons of slopes and intercepts among models (Analyses of Covariance) were conducted according to Zar (1984) to evaluate the significance of differences between sites and species.

We have made heavy use of graphical displays to interpret the regression results. This is because some important patterns were most easily identified graphically, including non-linearities, individual data points with undue influence, and uneven coverage of the data on the predictor variable axis. All of these detract from the regression's reliability, the interpretation of r^2, and statistical inference.

Confidence intervals and prediction intervals (shown in many of the following graphical displays) were developed to quantify the uncertainty regarding the regression model. Detailed discussions and specific formulae for confidence and prediction intervals at each predicted Y value can be found in Neter et al. (1985). The interval estimates at each level (h) of the predictor (Equations 1 and 2) are connected to form a band encompassing the entire regression line.

1-α confidence interval for $E(y_h)$:

$$\hat{y}_h \pm t_{1-\alpha/2,\, n-2} * \sqrt{MSE\left[\frac{1}{n} + \frac{(x_h - \bar{x})^2}{\sum(x_i - \bar{x})^2}\right]} \qquad (1)$$

1-α prediction interval for y_h:

$$\hat{y}_h \pm t_{1-\alpha/2,\, n-2} * \sqrt{MSE\left[1 + \frac{1}{n} + \frac{(x_h - \bar{x})^2}{\sum(x_i - \bar{x})^2}\right]} \qquad (2)$$

where

$t_{1-\alpha/2,\, n-2}$ = t distribution critical value

$E(y_h)$ = expected value of y at level h (mean)
$\hat{y}_h$ = predicted value of y at level h
x_h = value of the predictor at level h
MSE = mean square error

A conceptual, somewhat simplified, description of confidence and prediction intervals is as follows. Confidence intervals describe the uncertainty about the location of the regression line. In bivariate regression, a population of response values arises for a given value of the predictor. The regression line represents the mean response at each potential value of the predictor and the 95% confidence interval at a given predictor value represents the interval within which the mean response lies with 95% confidence. Uncertainty in estimating the mean, and therefore the confidence interval, becomes substantially narrower as the number of data points in the regression increases (note the 1/n term in Equation 1). The confidence interval flares at each end because uncertainty in estimating the regression line increases as the distance from the mean of the independent variable increases (note the numerator of the final term in brackets).

In contrast, the 95% prediction interval encompasses 95% of the population of response values expected to occur for a given predictor value. The prediction interval includes uncertainty in estimating the mean (center) of the population and therefore also decreases as N increases. However, as the confidence interval for the mean becomes very narrow, the prediction interval becomes dominated by the population variance (factor of 1 in Equation 2), which does not decrease with increasing N. The population variance is the same regardless of N, and although estimates of it will vary randomly with the sample data set, the estimates also do not decrease with N. For example, the 95 percent prediction interval at a given predictor point, X_h, is designed to encompass 95 percent of the response values. The 95 percent confidence interval is designed to encompass the true mean of these responses, 95 percent of the time.

Uncertainty Analysis

The uncertainty analysis performed in this study considers sources of variability[4] as well as uncertainty.[5] If the relationship between WS and MES varies by site and species, then this variability contributes to uncertainty in applying a single model to all sites and species. We provide evidence for such variability, incorporate it into an uncertainty analysis, and express it as a prediction distribution. In the Results section we demonstrate how the confidence interval approach substantially understates uncertainty when a single model, based on a single species and site, is applied to other species and sites.

We assumed relationships could include multiple regression lines, each characterizing different sites and species. In addition, scatter about each regression

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[4] Variability is defined as the inherent heterogeneity in the factor of interest.

[5] Uncertainty is defined as the imperfect information available regarding a defined value such as the mean of a distribution, or a regression relationship for a given site or species.

line was assumed to reflect a mix of measurement error and natural variation between ponds at a site. Because both response and predictor variables were defined as pond-wide means, we assumed the scatter was not attributable to variation in individual biota exposure and uptake, or micro-spatial variation in selenium distribution within a pond. Site-to-site variation (and possibly pond-to-pond variation) results from many factors that are confounded in the current data set (e.g., geochemistry, seasonal temperature fluctuations, food species composition, avian food factors, and spatial pattern of contamination). The site effect is also confounded with a study effect (e.g., different field study designs, sampling implementation, and lab analysis) which introduces both error and bias. All these factors cause uncertainty when combining data from different sites and when applying a single model to many sites. They cannot be distinguished in the data and so are collectively referred to as the site effect.

Random error associated with field sampling and laboratory analysis can be both site independent (i.e., inherent in all studies) and site dependent (i.e., increased for a particular study due to increased spatial variability, temporal variability, or other complexity in a site, increased detection limits, or reduced effectiveness of the field design and implementation). The latter source of random error probably dominates the former, and therefore, random error is best considered as a component of overall site variation (i.e., it contributes to model heterogeneity between sites).

The uncertainty in applying a global model based on the distribution of responses predicted at a given X_h, is characterized as the prediction distribution. The prediction interval encompasses 95% of this distribution. If the regression model had been characterized for a specific site and species and then used to predict future response values for this site and species, the site-specific prediction distribution would describe the between-pond variability and error.

It was not the intent of this paper to develop a specific model for a single site and species. Instead, we demonstrate the uncertainty in taking a site- and species-blind view, where the prediction distribution for a threshold WS value is calculated about a single, global, regression line fit to data from many species and sites. Variability within this distribution is hypothesized to be due to site and species effects, in addition to within pond variability and error.

The foregoing method for estimating uncertainty contrasts with the definition of uncertainty used by Skorupa and Ohlendorf. In applying their model to derive a waterborne selenium contamination threshold, they defined uncertainty as the uncertainty that would be associated with estimation of the single regression line and mean response for a given predictor level, for a particular site and species. This uncertainty was characterized by 95% confidence intervals. The 95% confidence band for estimating a model based on a single site and species is generally much narrower than that arising when applying a given model on a global basis, as will be demonstrated below.

In order to characterize the uncertainty in applying a global model to infer a waterborne selenium criterion for all sites and species, the inverse prediction distributions of the two component models (i.e., the distributions of predicting DS given MES and WS given DS) were incorporated. The inverse prediction distribution of X given Y is normal (for properly transformed data) and has parameters as given by Equation 3 (Neter et al. 1985):

$$E(X_h) = (y_h - b_0) \;/\; b_1 \tag{3}$$

$$S^2(X_h) = \frac{MSE}{b_1^2}\left[1 + \frac{1}{n} + \frac{(X_h - \overline{X})}{\Sigma(X_i - \overline{X})}\right]$$

A prediction distribution was developed on the WS values that could result in a MES of 20 mg/kg. The approach employed a two-phased simulation. First, 300 samples were drawn from the simulated prediction distribution of log DS given a log MES of 4.30 (i.e., 20,000 μg/kg). For each of these log DS samples, 300 additional samples were drawn from the prediction distribution of log WS given log DS. The combined set of 90,000 log WS samples defined the uncertainty distribution for log WS. This distribution is normal and becomes log normal (base 10) when transformed back to real space.

Relative to the 3 mg/kg "contamination threshold" used by Skorupa and Ohlendorf, and the 8 mg/kg threshold for effects on egg hatchability for American avocets and black-necked stilts used by Ohlendorf and Santolo, we considered 20 mg/kg to be a no-effects endpoint. This endpoint, the EC_{10} for mallard duck embryo teratogenesis, is more sensitive than the EC_{10} for either avocets or stilts. It appears to also be slightly less sensitive than the EC_{10} for the coot, although sufficient data to calculate an EC_{10} for coots were not available. After reviewing all of the laboratory endpoints that could be used to evaluate the potential for effects on bird populations, we considered this to be the most relevant and best supported by field data across multiple species.

RESULTS

Results of the data analysis are presented in four sections corresponding to the individual and combined transfer compartments in the food web model: waterborne selenium to food chain selenium (WS → FCS), waterborne selenium to estimated dietary selenium (WS → DS), estimated dietary selenium to mean egg selenium (DS → MES), and the full model (WS → DS → MES). These sections are similar to those used in Skorupa and Ohlendorf, and we continued their convention in defining food chain selenium (FCS) as the concentration in particular prey species, and dietary selenium (DS) as the concentration in a mixture of prey species in the avian diet. In each section, regression results are compared to the Skorupa and Ohlendorf model, and model variability between sites and species are discussed. Although tissue concentrations are always presented in units of mg/kg dry weight, the model formulas (coefficients) are scaled to correspond with tissue concentration in units of µg/kg to be consistent with Skorupa and Ohlendorf's model.

Waterborne Selenium → Food Chain Selenium Relationships

Skorupa and Ohlendorf presented regression models for a relationship between WS and FCS derived originally by Shelton (unpublished) for four invertebrate species. As discussed previously, Skorupa and Ohlendorf chose the brine shrimp model to represent the WS → DS component of their food web model. Accordingly, we compared this model to relationships observed for the invertebrate data sets gathered for this paper.

Figure 2 compares the brine shrimp model to the relationship observed for all invertebrate species and sites considered. The brine shrimp model has a similar intercept and a slightly shallower slope than the global model, implying somewhat less selenium bioaccumulation by the brine shrimp than the average for all species and sites in the data set.

Figure 2 incorporates a prediction interval for the combined data set and graphically illustrates how the use of a 95% confidence interval would be inappropriate for describing uncertainty. The 95% prediction interval encompasses almost two orders of magnitude. The 95% confidence interval for the global regression is very narrow relative to the data spread, in response to the large number of data points. This is because the confidence interval encompasses the uncertainty about a hypothetical "average" site. The 95% confidence interval is not designed to encompass variability for different species and sites. The brine shrimp model developed was based on Tulare Basin data and represents a model that was developed for a single species and site group. It falls outside the confidence interval, but is consistent with the prediction interval, which is interpreted to encompass numerous such models. Thus, the 95% confidence interval shown here gives a very misleading picture of the uncertainties in applying the global model to different species and sites.

Further evidence for species- and site-specific effects is seen by modeling species and sites with greater than four sample pairs (WS and FCS). Statistically significant (α=0.06) differences between sites can be seen for chironomids (San Joaquin vs. Benton, Figure 3) and corixids (San Joaquin vs. Kendrick vs. Benton, Figure 4), but not for odonates (Green River, Kendrick, San Joaquin Valley, not shown). Chironomids in the San Joaquin Valley had a steeper slope than chironomids at other sites while corixids had a slightly flatter slope than other sites (Table 3). Insignificant differences between sites for other invertebrate taxa are likely due to inadequate sample sizes.

Differences between species at a given site are shown for San Joaquin (Figure 5) and Benton (Figure 6). For San Joaquin, corixids had a significantly flatter slope than chironomids (slopes of chironomids, dipterans, and odonates did not differ significantly). For Benton, slopes for corixids and chironomids were similar, but intercepts differed.

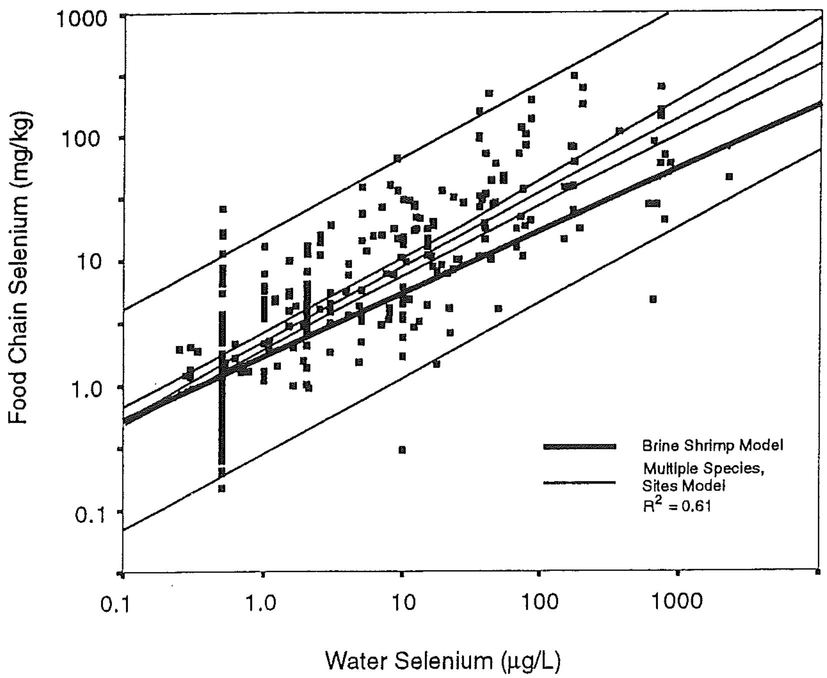

Figure 2. WS --> FCS: All Invertebrate taxa, All Sites

The slopes, intercepts, and respective confidence intervals of the regression lines are given in Table 3 for all cases with greater than 4 pairs. Statistical comparisons between the sites and species in Table 3 may be less reliable given apparent nonlinearities in the data (possibly due to censoring of low, nondetected WS values) and clustering of sites along the WS axis (e.g., Figure 3). Consequently, we have placed greater emphasis on the graphical displays to identify potential differences in food-chain bioaccumulation.

Differences between sites were not consistent among different species; it is likely that effects are also not constant across all species at a given site. Rather than detract from the importance of a site-specific or study-specific effect, this indicates site-specific studies of selenium bioaccumulation are necessary and emphasizes the need for a better understanding of the factors controlling its bioaccumulation.

The prediction interval bounding the full data set are shown with the site- and species-specific models in Figure 7. This figure suggests the range of mean FCS values that could arise from a given WS, depending on site and species. The prediction distribution at a given WS incorporates variability in the expected FCS (the regression lines) and also the scatter about each model. This scatter is due to differences between pools at a site, random error, and other factors such as seasonal differences. Because all four components (site variability, species variability, between-

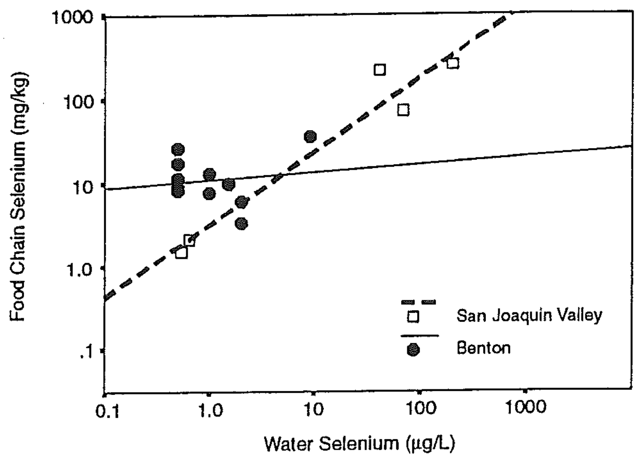

Figure 3. WS --> FCS:
Chironomids - Two Sites

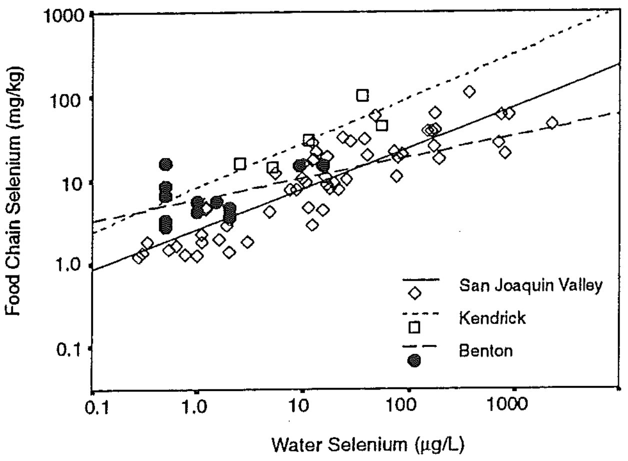

Figure 4. WS --> FCS: Corixids
- Three sites

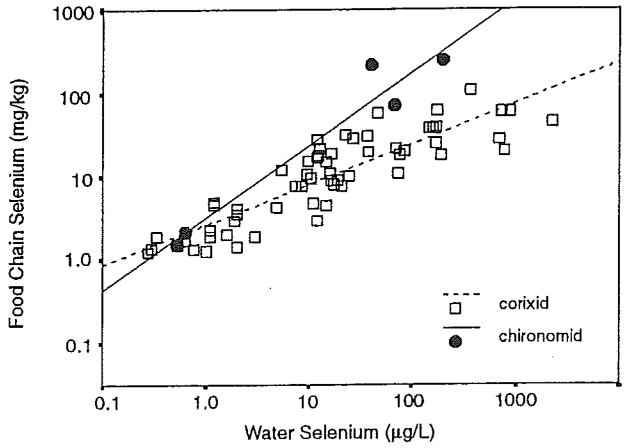

Figure 5. WS --> FCS: Chironomids
and Corixids - San Joaquin Valley

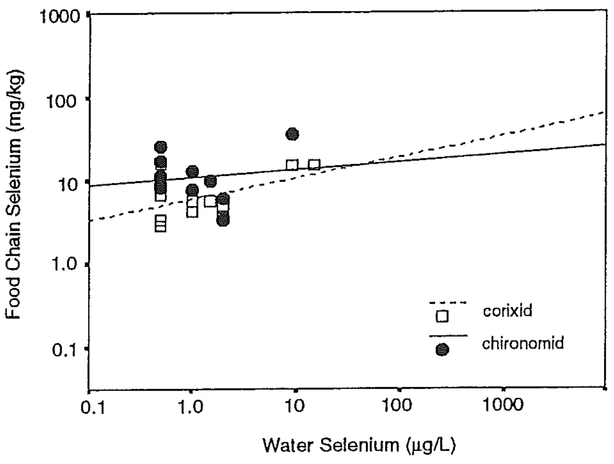

Figure 6. WS --> FCS: Chironomids
and Corixids - Benton

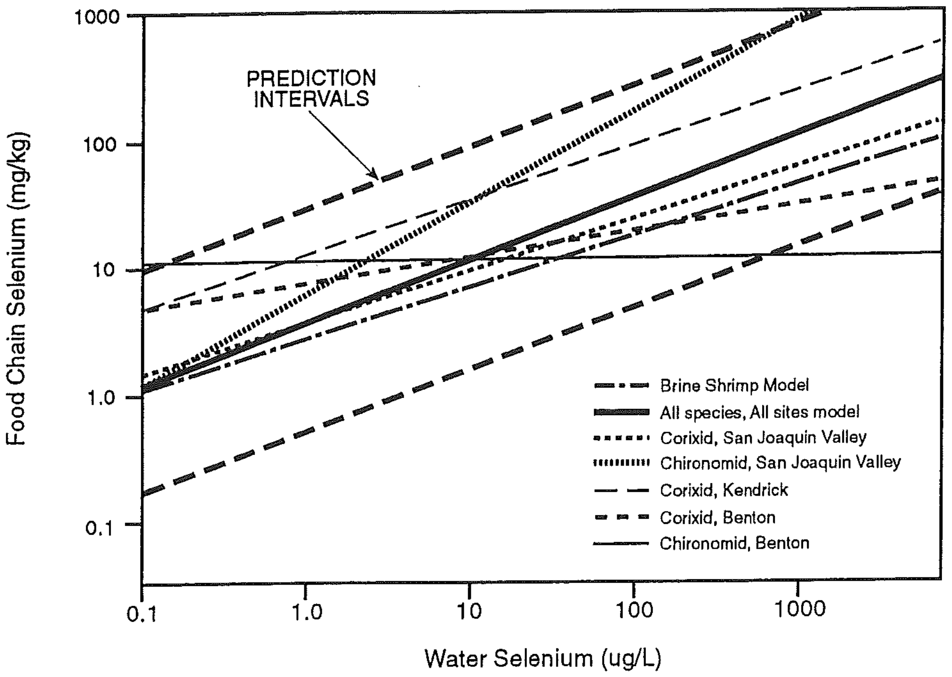

Figure 7. WS --> FCS: Corixids, Chironomids - Three Sites

pool variability, and random error) presumably contribute to the uncertainty in applying the global model to generic sites and species, we use the prediction interval and corresponding prediction distribution to describe the uncertainty in applying the global model.

Waterborne Selenium → Dietary Selenium Relationships

Site and species effects increase the importance of accounting for diet composition and spatial averaging in estimating dietary selenium exposure for avian species. Values of DS for eared grebe, avocet, stilt, mallard, and coot incorporated estimated dietary fractions obtained from published reports of field surveys. However, as discussed in the Methods Section, inconsistencies in the occurrence of food items in the data set implied that the actual dietary fractions contributing to a given DS value were influenced by the type of data collected at a site or pool rather than solely by the theoretical fractions. Thus, the WS → DS relationships established in this section are misleadingly similar for some species (e.g., avocet, stilt, and eared grebe) because they often reflect the same or similar DS values at a given location. The herbivorous bird species (coots and mallards to a lesser extent) had measured plant selenium concentrations for a high percentage of their DS, but species sampled may not correspond with species consumed.

TABLE 3--Summary statistics for log (WS) to log (FCS) models.

Area	Taxon	n	r^2	slope	lci[1/]	uci[2/]	p[3]	intercept	lci[1/]	uci[2/]	p[3]
Benton	Chironomid	11	0.02	0.089	-0.458	0.636	0.721	4.036	3.829	4.243	0.000
Benton	Corixid	12	0.23	0.251	-0.072	0.572	0.114	3.777	3.616	3.937	0.000
Green River	Chironomid	6	0.35	0.227	-0.201	0.655	0.215	3.799	3.380	4.218	0.000
Green River	Hemiptera	5	0.60	0.337	-0.170	0.843	0.125	3.662	3.088	4.237	0.000
Green River	Odanata	8	0.81	0.418	0.213	0.624	0.002	3.837	3.614	4.061	0.000
Kendrik	Corixid	5	0.73	0.530	-0.060	1.119	0.065	3.920	3.218	4.622	0.000
San Joaquin Valley	Chironomid	5	0.94	0.873	0.475	1.272	0.006	3.501	2.901	4.100	0.000
San Joaquin Valley	Corixid	60	0.77	0.486	0.417	0.556	0.000	3.417	3.313	3.521	0.000
San Joaquin Valley	Odonata	15	0.86	0.642	0.484	0.800	0.000	3.504	3.214	3.794	0.000
San Joaquin Valley	Diptera	24	0.67	0.710	0.490	0.931	0.000	3.237	2.918	3.556	0.000
All Sites	all taxa	291	0.61	0.589	0.535	0.644	0.000	3.382	3.321	3.443	0.000
Shelton	brine shrimp			0.490				3.250			

[1] lci = 95% lower confidence interval.
[2] uci=95% upper confidence interval.
[3] Probability that slope is 0.

The data sets used for estimating WS → DS for individual bird species produced extremely similar models, and similar scatter about the models, for all avian species. The largest difference was seen for the coot model, which had a steeper slope but slightly lower intercept than the other bird species. The following global model[6] was fit for all sites and bird species combined (Equation 4):

$$\log DS = 3.2345 + 0.6327 * \log WS \qquad (4)$$

Because of artificial similarities in bird diets induced by macroinvertebrate sampling methods which focused on corixids and chironomids, data variability about the global model is due primarily to site effects and not species effects. However,

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[6] Note: All terms in equations in this paper are expressed as μg/L for water and μg/kg for tissue. Tissue concentrations discussed in the text and presented in figures have been converted to mg/kg for ease in interpretation.

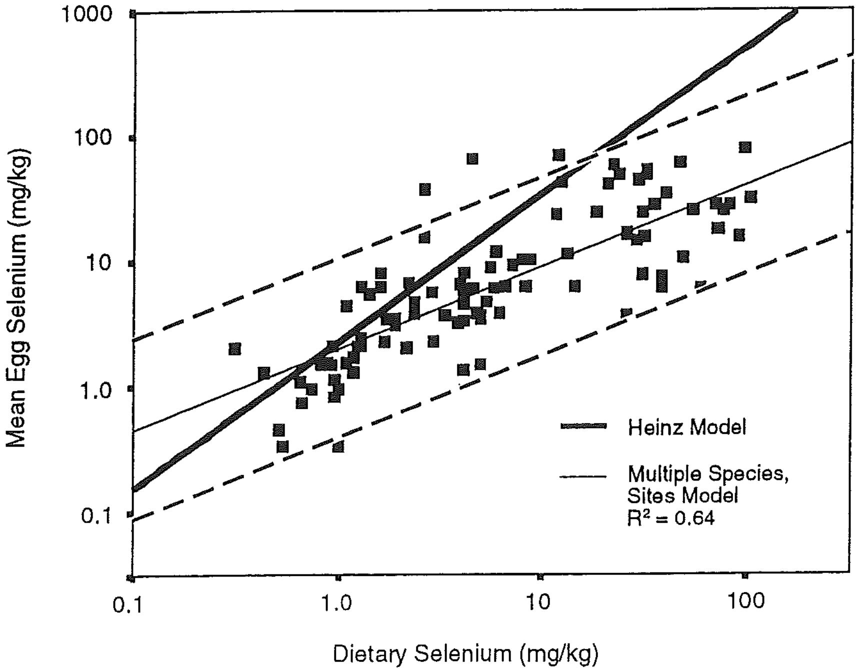

Figure 8. DS --> MES: All Species - All Sites Compared to Heinz et al. 1989

with more comprehensive data, there may be additional variation imparted to the global model due to variation in WS → DS across bird species.

Dietary Selenium → Mean Egg Selenium Relationships

The relationship between DS and MES is shown for all birds and sites in Figure 8, along with the DS → MES model used by Skorupa and Ohlendorf. The latter was based on a study of game-farm mallards (Heinz et al. 1989), and is referred to as the Heinz et al. model. Our global model for this relationship is (Equation 5):

$$\log MES = 0.7867 + 0.8241 * \log DS \qquad (5)$$

The Heinz et al. mallard model has a steeper slope than the global model and overestimates a large portion of the data (Figure 8). The difference in slopes suggests a strong species and/or study effect that can also be seen in Figures 9 and 10. The figures display the non-linearities present in some DS → MES relationships that reduce the power of the statistical tests and the reliability of findings of "non-significant" differences. For example, the coot model had a significantly lower intercept and steeper slope than the avocet, stilt, and mallard models, but the slopes

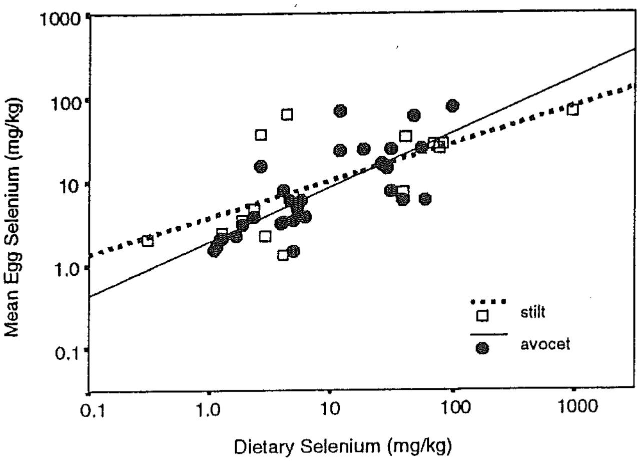

Figure 9. DS --> MES: Avocets, Stilts - All Sites

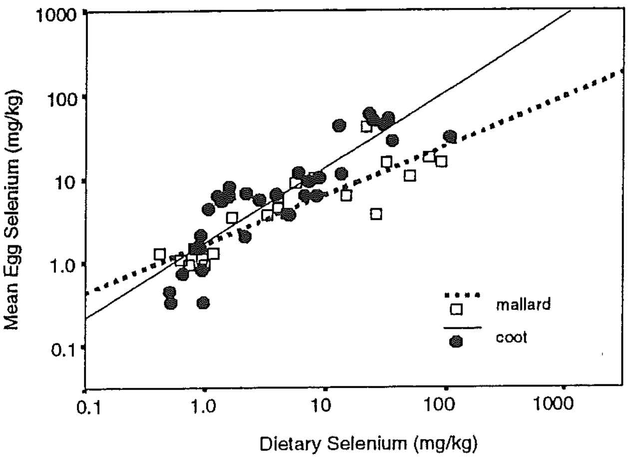

Figure 10. DS --> MES: Coots, Mallards - All Sites

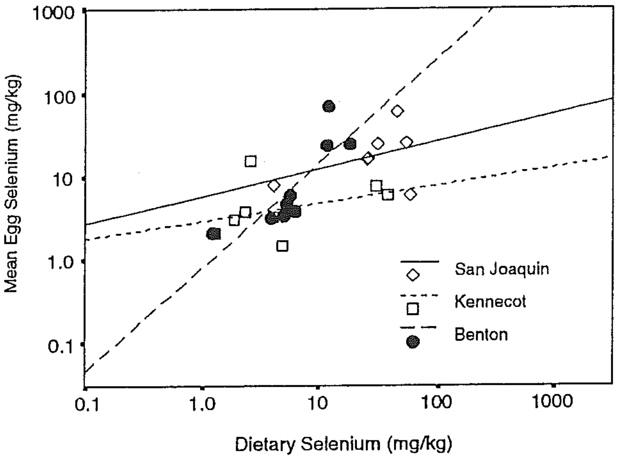

Figure 11. DS --> MES: Avocets - Three Sites

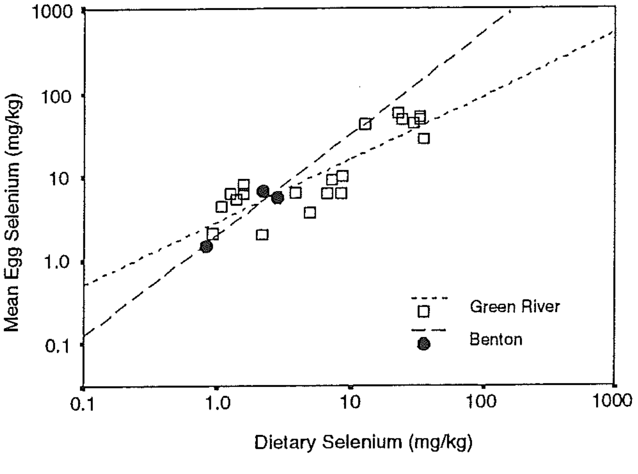

Figure 12. DS --> MES: Coots - Two Sites

and intercepts for the latter three species were not statistically different from each other. The coot model is most similar to the Heinz et al. model and suggests coots may bioaccumulate selenium in their eggs to a greater extent than stilts, avocets, or grebes when exposed to similar DS.

Significant differences in site-specific relationships were observed in the avocet data set for San Joaquin Valley, Kennecott, and Benton, the sites with at least four points (Figure 11). The coot data suggest similar relationships for the two sites with at least three points, Benton and Green River (Figure 12), but the statistical test for difference between these sites was inconclusive because of the small sample size for Benton (n=3). For the stilt and mallard data sets, investigation of the differences between sites was inconclusive because of relatively wide scatter or low sample sizes ($n \leq 3$).

All site- and species-specific models, and the prediction intervals bounding them, are shown in Figure 13. This figure illustrates the expected range of MES values that can arise from a given value of DS, depending on site and species. As explained above, the predicted distribution at a given value of DS incorporates variability in the expected FCS (the individual macroinvertebrate regression lines) and also the scatter about each model.

Waterborne Selenium → Dietary Selenium → Mean Egg Selenium Relationships

The global models for WS → DS and DS → MES, were combined algebraically into a two-step model for WS → DS → MES. The two-step composite model was (Equation 6):

$$\log \text{MES} = 3.5193 + 0.3947 * \log \text{WS} \tag{6}$$

In addition to the two-step model, a one-step global model, estimating WS directly from MES, was developed. This model has a very similar equation to the two-step model, but implies narrower prediction intervals and is therefore, more certain than the two-step model. The one-step composite model ($r^2 = 0.67$) was (Equation 7):

$$\log \text{MES} = 3.3660 + 0.5610 * \log \text{WS} \tag{7}$$

As a general principle, a food web model (i.e., 2 or more steps) is expected to have more uncertainty than a one-step model because error and variability enter the model at each trophic level in the food web, rather than only at the top consumer. The two-step model provides, however, additional information about bioaccumulation in the intermediate trophic levels and allows use of selenium concentrations, measured on-site in food items and water, for assessment and calibration. The one-and two-step models were compared to the Skorupa and Ohlendorf model (Figure 14). In general, there is reasonable agreement among the models themselves (i.e. aside from the characterization of uncertainty).

A review of the data shown graphically in Figure 14 reveals that variability in response existed at both ends of the regression line. Relatively low (background) WS levels (~ 0.5 μg/L) were associated with MES ranging from 0.5 to 5 mg/kg and relatively high WS levels (≥ 50 μg/L) were associated with MES that ranged from 5 to 100 mg/kg. This variability in the data (also reported by Skorupa and Ohlendorf 1991 in the data set used to determine the threshold for contamination (3 mg/kg)) indicates there may be site-specific factors accounting for selenium bioavailability and transport.

We used the global one-step and two-step models to develop ranges of water concentrations that would be protective of bird eggs for selenium of 20 mg/kg. (Table 5). For example, for 75% of the sites and species, a WS of 16.8 μg/L or lower would be protective of birds with an MES threshold of 20 mg/kg. For 50% of sites and species, WS values less than 128 would be protective of the 20 mg/kg endpoint.

The model outputs are not directly comparable to the results of Skorupa and Ohlendorf's model in that different thresholds were selected in each case. As noted previously, Skorupa and Ohlendorf used a MES threshold based on the contamination threshold derived in their paper (MES = 3 mg/kg), while the threshold of 20 mg/kg is the EC_{10} for mallard duck embryo teratogenesis (Skorupa et al., 1996).

The model developed in the present paper differs substantially from previous models in how uncertainty is represented. The threshold WS range associated with our model consists of a probabilistic prediction distribution across the range of sites evaluated (Table 5) instead of a range based on a point estimate of the mean and its corresponding upper 95% confidence limit. The output of the Skorupa and Ohlendorf model has been used to provide a point estimate of a selenium concentration in water that would be protective of bird-egg residues at a given threshold level (Skorupa and Ohlendorf 1991 and Ohlendorf and Santolo 1994). The data were derived from one site or area and extrapolated to other areas. The point estimate was generated from the lower 95% confidence interval about the regression mean from water to food-chain selenium and food-chain to bird egg selenium. Using the Skorupa and Ohlendorf model, a MES of 3 mg/kg resulted in an upper 95 percent confidence limit of 2.3 μg/L and a point estimate of 0.5 μg/L (Skorupa and Ohlendorf 1991) while a MES of 8 mg/kg resulted in an upper 95 percent confidence limit of 7.8 μg/L (Ohlendorf and Santolo 1994) and a point estimate of 2.7 μg/L. However, this does not imply that one would have 95% confidence that all sites were protected to the same extent. The degree of protection provided across sites is unknown. Our approach examined the variability across sites, and across species of birds and diets at those sites, and predicted the proportion of sites and species that would be protected by a given water selenium or a given dietary selenium concentration. This approach is one that has not been widely used and offers a unique assessment of the protection afforded across sites by water quality criteria.

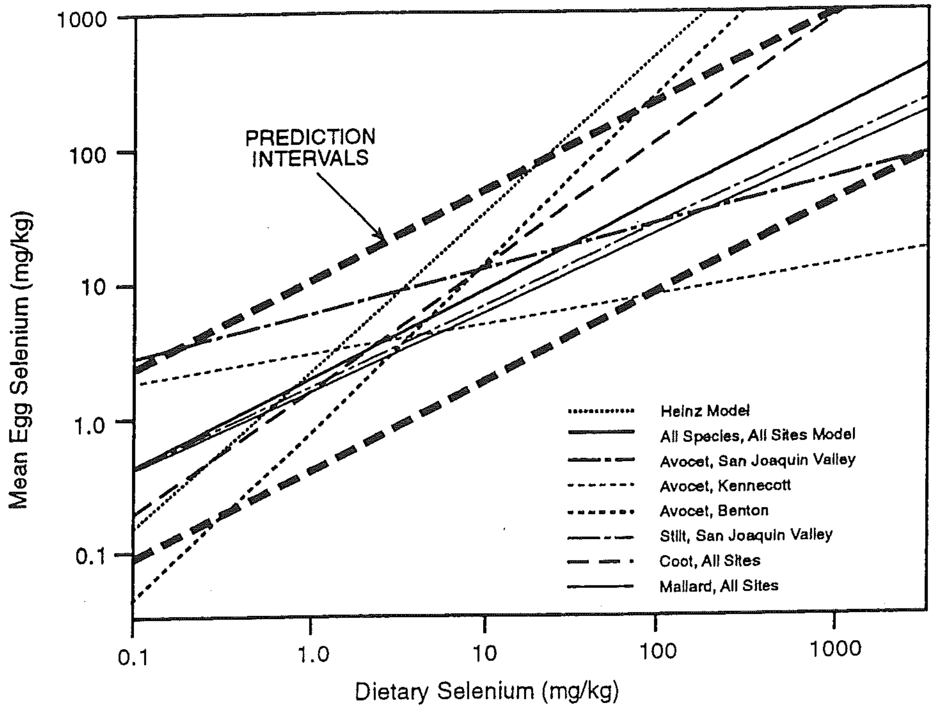

Figure 13. DS --> MES: Selected Species and Sites, Heinz et al. (1989) and Prediction Intervals for Global Model

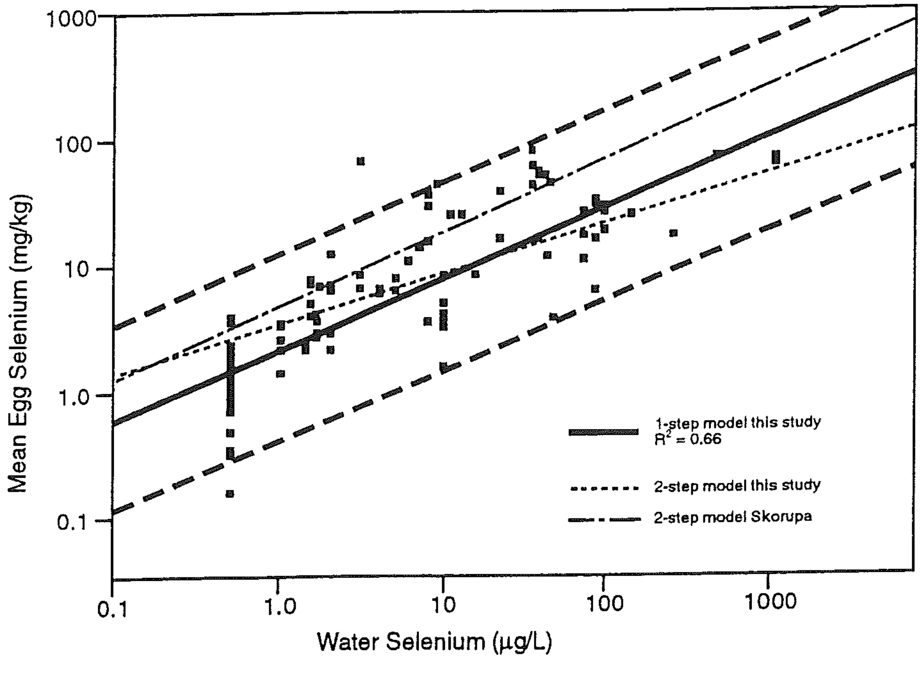

Figure 14. WS-->MES: All Bird Species and Sites, 1-step and 2-step models

TABLE 4--Summary statistics of log (DS) to log (MES) models.

Area	Species	n	r^2	lci	uci	p	slope	intercept	lci	uci	p
all sites	Avocet	30	0.58	0.434	0.858	0.000	0.646	1.352	0.517	2.188	0.003
all sites	Coot	33	0.79	0.726	1.069	0.000	0.897	0.547	-0.079	1.172	0.085
all sites	Mallard	21	0.77	0.428	0.733	0.000	0.581	1.45	0.913	2.058	0.000
all sites	Stilt	15	0.47	0.147	0.728	0.000	0.438	2.266	1.071	3.462	0.001
all sites	all birds	98	0.64	0.647	0.746	0.000	0.647	1.368	0.990	1.747	0.000
Heinz	Mallard			1.170				-0.142			

TABLE 5--Distribution of water selenium (WS) values that would protect a 20 mg/kg mean egg selenium (MES) threshold for different percentages of sites and species.

	Percent of sites and species protected				
MES (mg/kg dry weight)	90%	75%	50%	25%	10%
		WS (µg/L) Two-Step model			
20	8.88	17.6	94.9	510	2,320
		WS (µg/L) One-Step model			
20	6.77	16.8	46.4	128	318

DISCUSSION

The primary goal of the models developed by Skorupa and Ohlendorf (1991) and Ohlendorf and Santolo (1994) was to use field and laboratory data for deriving a predictive relationship between waterborne selenium concentrations and contamination/ adverse effects thresholds for birds. Our goal was the same, but we also wanted to include data from multiple sites and multiple bird and dietary species as opposed to one site, one bird species, and one dietary species. We also considered the variability that exists within the data sets and the model output. Our analyses suggest Skorupa and Ohlendorf's approach, though accurate for the specific conditions they examined, has limitations when extended to other sites. Accounting for the uncertainty in WS → DS → MES relationships through use of probabilistic techniques reveals more than an order of magnitude variability in predicting MES from WS, and vice versa.

Skorupa and Ohlendorf underestimated uncertainty from a global perspective because they considered only one site (San Joaquin Valley), one avian species (eared grebe), and one aquatic prey species (brine shrimp); it was not an explicit objective of their paper to develop a global model. Their model and exposure pathways, as well as their regression equation and predictions, are not mechanistically different from ours; they differ only in terms of the number of sites, species, and data uncertainties considered. Accordingly, the uncertainty in applying a broad-based selenium water quality criterion protective of wildlife across multiple sites is an order of magnitude or more larger than would be derived using a model based on one site. The magnitude of this uncertainty underscores the difficulty in ascribing a single number that is protective of all sites without choosing a value that is extremely low (perhaps as low as 0.5 µg/L). This is constrained by the fact that background levels of selenium often range up to 1-2 µg/L, or higher, depending upon the surrounding geologic formations.

Uncertainties presented in the present study represent differences among sites and species, which in turn may reflect differences in selenium forms for both WS and FCS and differences in the feeding strategies of their avian predators. Skorupa and Ohlendorf recognized these two factors as the most significant contributors to uncertainty. Species-specific differences exist in WS → FCS relationships, bioaccumulation in individual species varies across sites, and substantial differences in food habits of many shorebirds and waterfowl (both between species and within species across sites) create different selenium doses. Due to a paucity of data on selenium residues in the same species of bird feeding on different prey and an overall lack of information on the specific food items consumed by each species at each site, our analysis was unable to distinguish the relative importance of diet composition and the amount of selenium derived from various food prey items.

Overall, our analysis indicates a strong correlation between water and mean egg selenium concentrations and suggests a useful relationship for assessing selenium's potential to reach established effects thresholds in bird eggs. However, site-specific factors strongly influence this relationship such that a water quality criterion of 2 µg/L should be considered useful only as a screening level tool for assessing potential effects on bird populations. When WS approaches or exceeds this level, the model should be calibrated with site-specific data to more accurately determine the site-specific WS → MES relationship. This relationship can then be used to decide whether site-specific differences are important and, if so, to derive a site-specific WS value that would be protective of bird populations without being overly conservative.

Using the uncertainty analysis from our one-step model, a 90th percentile value of 6.8 µg/L WS is associated with a threshold concentration (20 mg/kg in the egg) for reproductive effects in birds. If the approach to developing a water quality criterion were to be based on a global model, using a value such as the 90th percentile to derive the threshold WS concentration would appear to be appropriate. The 90th percentile estimated in this study (6.8 µg/L) is greater than the 2.0 µg/L value recommended by the USFWS (Lemly and Smith 1987, Lemly 1993, 1995, 1996) for wildlife protection. However, it is comparable to the current 5 µg/L national chronic ambient water quality criterion for protection of aquatic life. The difference between the 2.0 and 6.8 values is due to differences in the models and differences in the endpoints chosen to be protected, e.g., contamination threshold (Skorupa and Ohlendorf 1991) and reproductive effects threshold (present paper).

The models developed here provide flexibility in estimating selenium residues in bird eggs using data on either waterborne or food chain selenium. In applying the model to a site in which both WS and FCS data are available, and are adequately co-located in time and space, DS could be estimated and used to develop a site-specific model equation for WS → DS. Comparison of such site-specific models to the global model developed here would provide better insight into the relative importance of dietary composition when developing a site-specific criterion. However, such an analysis would still not address the question of how to predict MES with few or no site data on MES. In these situations, a one-step WS → MES regression will always provide a better fit to the field data than a two-step model. Methods for estimating DS (which depend on FCS field data, feeding fractions, and assumed spatial averaging) are more variable, in the aggregate, than methods used to estimate MES from WS, and the direct least squares regression of the log WS on log MES calculates the regression line that minimizes the prediction error for MES. A one-step model will give the highest r^2 achievable for a given data set.

Overall, to determine the potential for reproductive effects in birds, we recommend use of field data on site-specific MES, rather than predictions based on either water concentrations or invertebrate tissue levels. Bird egg selenium concentrations provide a useful screening tool for potential reproductive effects; however, confirmation of effects, such as impaired hatchability, may be needed and should be obtained by field evaluations.

Selenium is recognized as a substance with a small effects concentration to background concentration ratio that makes it difficult to resolve WS → DS → MES relationships. Current analytical capabilities, biological variability in responses, and lack of studies designed to measure these relationships make it difficult to resolve the various components of variability and uncertainty. For example, the approach discussed here does not address the sediment compartment, the mass of selenium cycling within a given ecosystem, or the retention time (flushing rate) of a given aquatic system. Canton and Van Derveer (1997), and Van Derveer and Canton (1997) have found that lotic systems (flowing water) can have much higher selenium levels without apparent effects on aquatic life compared to lentic (wetland) systems. The difference in exposure effects concentrations may be attributed, in part, to the fact that lotic systems are more aerobic, contain predominantly selenate as opposed to the reduced selenium forms which predominate in lentic systems, and have more rapid flushing times. These two studies have proposed a lotic model for predicting the concentration of selenium in water that is protective of aquatic life that considers the interaction of total particulate organic carbon and selenium concentration. While this approach has not been applied to wetland ecosystems, it supports the hypothesis that the sediment compartment plays an important role in determining the fate and transport of selenium. Additional research is needed on these and other questions to resolve relationships of selenium transport in ecological systems.

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