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Egg Selenium Concentrations as Predictors of Avian Toxicity

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ABSTRACT

Aquatic birds are exposed to selenium through their diet by ingesting aquatic invertebrates that have accumulated selenium from water and the food chain. However, dietary composition is highly variable among species, over time, and across sites, making it difficult to provide accurate estimations of dietary exposure for particular species at specific locations. Selenium accumulates in the egg, resulting in embryo malformation, embryonic death, and decreased survival of juveniles. If the relationship between egg concentration and these reproductive parameters can be defined with sufficient certainty, then risk assessments can be performed through analysis of egg selenium concentrations. Other researchers have proposed egg toxicity thresholds that lead to conclusions of widespread selenium toxicoses in waterbirds. However, we believe these values are overly conservative and that it is unlikely that selenium is posing a significant risk to wild birds in areas where the current water quality criterion is being met. Through the use of simple statistical models (logit, probit, and Weibull functions) we are able to express mortality and teratogenicity relationships for mallards (*Anas platyrhynchos*) in such a manner that the risk manager can be presented with information about the probability of reduced duckling survival if mean egg selenium (MES) concentrations are known. Data analysis indicates that the two endpoints (mortality

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and teratogenesis) cannot be distinguished statistically. Commonly used effects thresholds (the EC_{10} and EC_{20}) correspond to 16 and 21 mg/kg dw MES using the most sensitive endpoint, chick mortality. Both of these values are higher than the 6 mg/kg dw level proposed by Skorupa (1998) who based his estimate on field-observational data potentially confounded by other environmental stress factors. The mortality and teratogenicity endpoints presented here relate selenium exposure to risk to individuals within a population and do not provide information about the probability of selenium causing changes in population growth rates, either at the local or regional levels.

Key Words: selenium, birds, toxicity thresholds

INTRODUCTION

Studies conducted with poultry in the 1930s and ducks in the 1980s and 1990s conclusively demonstrated that birds are sensitive to the toxic effects of selenium-contaminated feed (Poley and Moxon, 1938; Poley *et al.*, 1941; Heinz *et al.*, 1987; Heinz, Hoffman, and Gold, 1988, 1989; Hoffman and Heinz, 1988; Heinz and Fitzgerald, 1993; Stanley *et al.*, 1994). These studies suggest a reproductive effects threshold of about 5 mg/kg-dry weight (mg/kg dw) in the diet for sensitive bird species, such as mallards (*Anas platyrhynchos*) (Table 1). Indeed, selenium poisoning of wild birds was documented at Kesterson National Wildlife Refuge in the mid-1980s when agricultural drainwater raised the water concentrations to an average of 300 μg/L and the invertebrate selenium concentrations to levels much greater than the 5 mg/kg dw avian toxicity threshold. Some researchers have suggested that similar toxicoses are occurring at many other wetland sites in the arid west, based on exceedences of proposed egg threshold concentrations for selenium. This paper reviews the basis for the determination of these thresholds and concludes that they are overly conservative, therefore rendering the predictions of widespread selenosis incorrect. In reality, only a few sites exceed the more reasonable thresholds proposed herein, all of which also exceed the USEPA water quality criteria of 5 μg/L. Consequently, it is unlikely that selenium will pose a significant risk to wild birds when the current water quality criterion is being met.

Predictions of toxicity to birds based on measurements of either water or dietary concentrations can be very difficult (Adams *et al.*, 1998). The composition of the diet of aquatic birds varies over time and across sites. Most birds are opportunistic feeders and an invertebrate hatch or windrow will cause them to exclusively consume a very abundant species. Furthermore, water birds forage extensively across areas that generally are not uniformly contaminated. These factors make it difficult to develop a reliable measure of dietary exposure. Composite collections of invertebrates provide uncertain exposure estimates for any particular population of interest, contributing to uncertainty in risk estimates based on dietary exposure concentrations.

Dietary composition also varies across wetland birds species. For example, Euliss and Jarvis (1991) reported that pintails (*Anas acuta*) consume about

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Table 1. Dietary toxicity threshold for birds exposed to selenium in laboratory studies.

Species	Endpoints measured	Selenium form	Effect level (ppm dw) NOAEL	Effect level (ppm dw) LOAEL	Ref.
Chicken	Egg hatchability	Seleniferous grain	2.5	5	Poley and Moxon, 1938; Poley *et al.*, 1941
Japanese quail	Hatchability (as % of fertile)	SeMet		5	Martin, 1988
Mallard	Onset of egg production Laying interval Hatch successes Hatch weight	Selenite		25	Heinz *et al.*, 1987
	Onset of egg production Laying interval Hatch successes Hatch weight	SeMet		10	
Mallard	Embryo malformation	Selenite	5	10	Hoffman and Heinz, 1988
	Hatchability		10	25	
	Embryo malformation	SeMet	4	8	
	hatchability		4	8	

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Table 1. Dietary toxicity threshold for birds exposed to selenium in laboratory studies. (continued)

Species	Endpoints measured	Selenium form	Effect level (ppm dw) NOAEL	Effect level (ppm dw) LOAEL	Ref.
Chicken	Egg hatchability	Seleniferous	2.5	5	Poley and Moxon, 1938;
Mallard	Embryo malformation	SeMet	4	8	Heinz *et al.*, 1989
	Hatchability (as % of fertile)		8	10	
Mallard	Embryo malformation Hatchability (as % of fertile)	SeCys	16		Heinz *et al.*, 1989
Mallard (juveniles)	Subchronic toxicity (mortality)	Selenite	20	40 (25% dead)	Heinz *et al.*, 1988
		SeMet		40 (12% dead)	
				80 (100% dead)	
Mallard	Adult mortality Body weight	SeMet		20	Heinz and Fitzgerald, 1993; Heinz *et al.*, 1987

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Mallard	Hatching success	SeMet	10 (8.5%)		Stanley *et al.*, 1994
	Embryo malformation		10 (57.5%)		
	Duckling Mortality	SeMet	10 (90%)		
Black crowned night heron	Reproduction				
	Body weight	SeMet	10	30	Smith *et al.*, 1988

SeMet = selenomethionine.
SeCys = selenocysteine.

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50% of their diet as plant matter and that midges (Chironomidae) dominate the animal portion of their diet. Water boatmen (Corixidae), rotifers (Rotifera), and copepods (Copepoda) are the most commonly consumed foods by shovelers (*A. clypeata*), which have less than 10% of their diet comprised of plants. Ruddy ducks (*Oxyura jamaicensis*) consume midges and water boatmen with only a few additional species of invertebrates and about 10% plants. However, these diets vary greatly over the winter and spring seasons, from 100% plant material in pintail diets in October to greater than 80% animal foods in March.

While dietary exposure to selenium is difficult to measure in the field, selenium has another attribute that lends itself to exposure estimation. This is the property of accumulation in the egg, which can result in embryo malformation (teratogenicity) and chick mortality when sufficiently high concentrations are reached. Therefore, selenium risk assessments can be performed through analysis of mean egg selenium (MES) concentrations rather than attempting the more uncertain task of correctly estimating dietary exposures.

In general, three methods historically have been used to set MES risk thresholds for birds: contamination thresholds, no observed adverse effect levels (NOAELs), and exposure-response functions. We will discuss each in turn, then focus more closely on exposure-response functions, which we believe provide the best approach for assessing risks to birds. We considered two toxicological endpoints — chick mortality and teratogenicity.

For purposes of this paper, mortality is defined as the combined effects of embryo teratogenesis and mortality, hatchability and chick survival on net bird productivity. Laboratory data are available to develop mallard (*Anas platyrhynchos*) exposure-response functions for mortality, as well as selenium-induced embryo teratogenicity. Equivalent laboratory data are not available for other species such as avocets (*Recurvirostra americana*) and black-necked stilts (*Himantopus mexicanus*) that commonly are encountered in Western U.S. wetlands. These birds, as well as mallards, have field-observational MES-teratogenicity and MES-mortality exposure-response data. However, field-based mortality data are of questionable reliability because mortality is not a selenium-specific endpoint; many other factors, such as disease, temperature, parental nutrition and other contaminants potentially confound these data. Therefore, we chose to place primary emphasis on mortality and teratogenicity exposure-response functions using data from mallard studies performed in the laboratory.

EGG SELENIUM CONTAMINATION THRESHOLDS

Skorupa and Ohlendorf (1991) summarized egg selenium data from 74 bird populations inhabiting non-marine wetlands they thought to be uncontaminated with selenium, in an effort to establish an egg selenium contamination threshold. The intent of establishing this threshold was to back calculate a water concentration that would protect against MES concentrations exceeding the contamination threshold.

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Unfortunately, only three of the data sets used by Skorupa and Ohlendorf (1991) are published in the peer reviewed literature. The balance of their data come from unpublished reports, presentations at meetings, or personal communications that have not been made available to the broader scientific community. This makes it difficult to assess the quality of the data. Questions such as how the eggs were selected for analysis (*e.g.*, systematic versus opportunistic), analytical method used, what species are included in the data sets, and how a "reference" site was defined are unanswered. Additionally, the MES reported for each site represented in the Skorupa and Ohlendorf (1991) database depends on the species mix of bird eggs collected at that site.

The question of how a "reference" site was defined is of particular concern, because the maximum environmental selenium concentration and the type of environment from which the eggs were collected can significantly affect the amount of selenium in an egg (Adams *et al.,* 1998). Where the line is drawn between "reference" and "contaminated" sites is still the subject of considerable debate. Selenium is a naturally occurring substance and is an essential micronutrient for plants and animals. It is present in various amounts in soils, from highly seleniferous areas to those that are considered selenium-deficient for agriculture or livestock. Therefore, discriminating a naturally high concentration from anthropogenic contamination can be difficult.

Assuming the unanswered questions can be answered satisfactorily, the Skorupa and Ohlendorf (1991) database indicates that for the 74 sites studied, MES is less than 3 mg/kg dw 90% of the time, although individual eggs ranged as high as 8 mg/kg dw, presumably without effect. Skorupa and Ohlendorf (1991) termed this "contaminant threshold value" a normal background index. The normal background index was later lowered to 2 mg/kg dw without further explanation (Skorupa, 1998). Additionally, Skorupa (1998) compared the 2 mg/kg dw value with individual egg selenium concentrations rather than MES. A more appropriate approach would have been to compare the normal background index with a site's MES, because the index value is a mean, or to have used the 90th percentile value of all data points in the index with the individual egg value for the site. We point out that the 90th percentile for all data points would be higher than 2 or 3 but less than 8 mg/kg dw. Because of the questions and problems described above, the Skorupa and Ohlendorf (1991) database seems limited in its utility to assessing in very general terms whether a site is similar to the reference sites they used in generating the background index value provided the MES concentration is calculated for all species across the entire site.

Even if the problems with the normal background index were resolved, its use as a threshold for risk assessment would be inappropriate. Hazard analysis in the risk assessment process is built on an understanding of the exposure-response relationship and knowledge of when concentrations become high enough to cause adverse effects. An increase above "normal background" does not necessarily translate to adverse effects. For nutrients, such as selenium, there is a range of exposure concentrations that is acceptable and a range of concentrations that can be compensated for by physiological homeostatic

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mechanisms prior to the onset of toxic responses (Ennever, 1994). The large range in MES collected from the presumed uncontaminated sites in the Skorupa and Ohlendorf (1991) database suggests that selenium has such a homeostatic mechanism. For characterizing risk, it is necessary to understand the relationships between MES and embryo response (*e.g.*, developmental effects, hatchability, and subsequent chick survival). The range of acceptable concentrations and the range of MES concentrations where compensation occurs have not been well defined for bird eggs. However, the toxicity portion of the dose-response range has been studied and is discussed in the following sections.

HYPOTHESIS TESTING BASED THRESHOLDS

Toxicity thresholds can be developed using hypothesis testing to analyze data sets. The No Observable Adverse Effect Level (NOAEL) is the maximum MES level in experimental studies with responses that are not statistically significantly different from the responses of the control group (*e.g.*, Bailer and Oris, 1997). The NOAEL is a better tool than the contamination threshold, but it too has disadvantages (Chapman, Caldwell, and Chapman, 1996). Among these:

- The NOAEL is limited to the discrete exposure levels tested in experiments, creating an underestimation bias in the effect threshold;
- The NOAEL tends to decrease as experimental precision (and therefore statistical power) increases, creating an over- or underestimation bias in the effect threshold depending on the study's statistical power;
- The NOAEL defines the highest concentration at which no effects are observed. The lowest observed adverse effect level (LOAEL) defines the lowest concentration at which effects are observed. Neglecting for the sake of simplicity the statistical power issue, the true effect threshold generally lies somewhere between the NOAEL and LOAEL.

Heinz *et al.* (1989) found that prevalence of teratogenesis and chick mortality increased significantly in ducks fed $\geq$8 mg/kg dw selenium as selenomethionine. At 8 mg/kg dw dietary exposure, MES was measured as 11 mg/kg-wet weight (ww) or 36.7 mg/kg dw, assuming an egg moisture content of 70% (as reported in Heinz *et al.*, 1989 and Ohlendorf *et al.*, 1986). At 4 mg/kg dw dietary exposure (the calculated dietary NOAEL for reproduction), the predicted egg selenium is 11.3 mg/kg dw. Thus, the embryo teratogenicity threshold lies somewhere between 11 and 37 mg/kg dw in mallard eggs. In deriving water quality criteria, USEPA estimates the threshold where chronic exposure will result in actual effects (termed the Chronic Value) as the geometric mean of the NOAEL and LOAEL (Stephan *et al.*, 1985). The geometric mean of the Heinz *et al.* (1989) NOAEL and LOAEL is 20 mg/kg dw MES. Interestingly, the Chronic Value of 20 mg/kg dw exactly corresponds

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with the estimated EC_{10} for mallard teratogenesis based on field data collected in the California central valley (Skorupa, Morman, and Sefchick-Edwards, 1996). However, as with the NOAEL and LOAEL, the Chronic Value is dependent on the statistical power of the study. This uncertainty in where the true effects threshold lies leads to uncertainty in estimating risk. Additionally, the NOAEL, LOAEL, and Chronic Value are indicators of statistical, not necessarily biological, significance. Consequently, use of full exposure-response relationships provides a more accurate and descriptive estimate of risk. This approach is discussed further below.

EXPOSURE-RESPONSE FUNCTIONS

While the NOAEL or Chronic Value often are used as simple estimators of an effect threshold, not all animals respond similarly to the same exposure levels. Thus, it is preferable to understand the entire dose-response relationship in order to describe the proportion of animals expected to respond at any given exposure level and the rate of increase in response as a function of increasing exposure. Exposure-response functions are more useful in risk assessment as they allow a probabilistic risk estimation and avoid the use of an arbitrary threshold as the point where risk begins.

Efforts to develop dose-response relationships between egg selenium concentrations and reproductive effects began with the studies at Kesterson National Wildlife Refuge (NWR) in California, a highly contaminated site with water concentrations averaging 300 μg/L. Overt teratogenesis of water bird embryos was widespread and immediately obvious to observers. Terata such as anophthalmia (missing eyes), incomplete beak development, brain defects (hydrocephaly and exancephaly), foot defects, and other gross anomalies were well documented (Hoffman, Ohlendorf, and Aldrich, 1988; Hoffman and Heinz, 1988) and easily observed in both field and laboratory studies by personnel with minimal training. More subtle teratogenic effects such as edema, stunted growth, enlarged hearts, liver hypoplasia, and gastroschisis also can be documented, but take considerably more effort and require highly trained personnel. Therefore, it is assumed that the dose-response relationships between egg selenium and embryo teratogenesis described in the field studies below were based on observations of gross abnormalities.

Field studies of embryotoxicity were conducted for a number of water bird species at Kesterson NWR. Ohlendorf *et al.* (1986) investigated the relationship between individual egg selenium concentrations and the predicted incidence of embryotoxicity in coots (*Fulica americana*) and black-necked stilts. Early studies showed coot embryos had an estimated 50% probability of embryo death or deformity when egg selenium concentrations were approximately 18 (95% C.I. 0 to 25) mg/kg dw. For stilts, the 50% embryotoxicity level occurred at 24 (95% C.I. 0 to 100) mg/kg dw and the 20% effect level (*i.e.*, EC_{20}) was approximately 5 (95% C.I. 0 to 15) mg/kg dw. However, these values should be treated with caution as they included samples that were nonrandomly selected for selenium analysis because effects were observed in the embryos

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(Skorupa *et al.,* 1996). Consequently, these data will over-predict effects. This is demonstrated by the EC_{20} for stilts (and presumably the more sensitive coots) which overlaps considerably with the range of "background" egg concentrations presented in Skorupa and Ohlendorf (1991).

Skorupa *et al.* (1996) summarized field information on embryotoxicity in ducks generated by DuBois (1988) from a wildlife refuge in Montana, and in stilts and avocets from Kesterson NWR, Tulare Basin, and more than 40 additional sites in California studied from 1983 to 1995. The data demonstrate duck embryos are more sensitive to selenium effects than are stilts and that avocets are less sensitive still (Table 2). However, only the studies from Kesterson NWR have been reported in the peer reviewed literature. The data quality from all the other studies cannot be verified and, therefore, although the information is interesting, we recommend that it not be used to set toxicity thresholds for regulatory purposes until it has been published in the peer reviewed literature. The field data for selenium-induced teratogenicity in ducks are, however, generally consistent with the relationships established in laboratory studies.

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Methods for Evaluating Sensitivity of Teratogenicity and Mortality Endpoints for Mallards

As noted above, field-observed MES-teratogenicity data are available for water bird species, including mallards, avocets, and black-necked stilts (Skorupa *et al.,* 1996), but not MES-mortality data. Heinz *et al.* (1989) and Stanley *et al.* (1994) provide data that can be used to develop mallard exposure-response

Table 2. Observed probability (%) of overt embryo terata for given concentrations of egg selenium

Egg selenium concentration (mg/kg dw)	Ducks	Stilts	Avocets
00–10	0.0%	0.4%	0.0%
11–20	3.2%	1.3%	
21–30	8.7%	5.0%	
31–40	40.0%	24.4%	
41–50	Insufficient data		3.8%
51–60	Insufficient data	74.4%	
61–70	Insufficient data		7.1%
71–80	100%	100.0%	
81–100	—	—	9.1%
101–120	—	—	50.0%

(from Skorupa *et al.*, 1996).

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functions for both teratogenicity and mortality. A third study, Heinz *et al.* (1987) also provide limited data on this relationship. However, the bioaccumulation factor between diet and eggs for the Heinz *et al.* (1987) study is quite different from what is observed in other laboratory and field studies (Adams *et al.,* 1998; Heinz *et al.,* 1989; Stanley *et al.,* 1994). This introduces significant variability and uncertainty into the MES-teratogenicity / mortality relationship while only providing two additional data points (a control and one test concentration). Consequently, this study has not been included in the following analysis.

Exposure-response data from Heinz *et al.* (1989) and Stanley *et al.* (1994) are shown in Table 3. The mortality data from these studies include control mortality of 42 and 25% respectively, indicating that some mortality was not selenium induced. We used the data presented in Table 3 to derive MES exposure-response functions for teratogenicity and mortality. We used three different models (logit, probit and Weibull) to explore the consequences of various alternatives for assessing the exposure-response relationship. Because there was significant mortality in the controls, we used Abbott's formula (Finney, 1971) to normalize the data prior to developing the models. Data were normalized for the individual studies prior to pooling them for analysis (Table 4).

There is considerable uncertainty between the two studies at an MES of approximately 37 and 42 mg / kg dw, with control-adjusted chick mortality rates of 51 and 99% for the Heinz and Stanley studies, respectively. Consequently, we also did a sensitivity analysis on the influence of these two data points on the dose response relationship. This analysis involved rerunning the three models, excluding first the 37 mg / kg dw MES treatment from Heinz *et al.* (1989), and then the 42 mg / kg dw treatment from Stanley *et al.* (1994) to see how the dose response curve was affected.

In addition to these analyses, we compared the MES-teratogenicity relationship, as measured by Heinz *et al.* (1989) and Stanley *et al.* (1994), to the MES-mortality exposure-response functions to determine whether the data are sufficient to statistically conclude that mortality is a more sensitive endpoint than teratogenicity. Similar to the above analyses, we needed to address the mortality observed in the controls prior to analysis. Use of Abbott's formula when making direct comparisons between dose-response relationships is not appropriate because normalizing the data alters the shape and slope of the dose-response. Consequently, rather than using Abbott's, we simply subtracted the control mortality from the treatment responses prior to performing the analysis. Data from each study were adjusted based on the control response observed in that study, prior to pooling the data for analysis.

To compare the MES-teratogenicity and MES-mortality responses, we tested whether the data were sufficient to statistically distinguish the slopes or intercepts of logistic regression models. A mortality slope greater than the teratogenicity slope would indicate some additional mechanism independent of teratogenicity was contributing to the mortality response. Indistinguishable slopes would be consistent with the hypothesis that the same mechanism(s)

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Table 3. Results reported in Heinz *et al.* (1989) and Stanley *et al.* (1994).

	Egg selenium (mg/kg dw)[1]							
	Heinz *et al.* (1989)						Stanley *et al.* (1994)	
Measurement	Control	2.8	5.3	11.3	36.7	60	Control	42
% fertility of eggs	98.6	96.3	97	97.9	96.6	99.6	100	100
% hatch of fertile eggs[2]	59.6	70.7	60	53.4	36.9	2.2[a]	91.4	8.5[c]
% survival of ducklings to 6 days of age	99.4	97.7	98.9	99.7	80.6[a]	0[a]	82.5	10[c]
% teratogenesis	0.6	0.9	0.5	1.4	6.8[b]	67.9[b]	0	57.5[c]

Note: [1]Dry weight egg concentrations were estimated assuming moisture content of 70% (from study).

[2]To 6 days for Heinz *et al.*, and to 14 days for Stanley *et al.*

[a] Significantly lower than controls ($p < 0.01$)

[b] Significantly greater than controls (significance level not stated).

[c] Significance relative to control responses not reported.

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Table 4. Heinz *et al.* (1989) and Stanley *et al.* (1994) data normalized for control response.

	Egg selenium (mg/kg dw)							
	Heinz *et al.* (1989)						Stanley *et al.* (1994)	
Measurement	Control	2.8	5.3	11.3	36.7	60	Control	42
Raw Results								
% mortality of ducklings to 6 days of age based on all eggs[a]	41.6	33.5	42.4	47.9	71.3	100	24.6	99.2
% teratogenesis based on all eggs[b]	0.2	0.3	0.2	0.7	4.4	66.4	0	52.6
Normalized for Control Response								
% of all eggs not resulting in a 6–day-old duckling	0	0	1.5	10.8	50.5	100	0	99.0
% teratogenesis	0	0.04	0	0.42	4.1	66.3	0	52.6

[a]Calculated as % fertility × % hatch of fertile eggs × % survival of ducklings.

[b]Calculated as [1–% fertility × % hatch of fertile eggs)] × % teratogenesis.

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responsible for teratogenicity are responsible for MES mortality (*e.g.*, Sprague, 1969). A mortality intercept greater than the teratogenicity intercept, but similar slopes could indicate mortality was possibly associated with subtle (*e.g.*, internal) teratogenic effects (Hoffman and Heinz, 1988) not measured by Heinz *et al.* (1989) or Stanley *et al.* (1994).

We compared the slopes and intercepts using the technique of Oris and Bailer (1997) where:

$$\text{logit}(p) = B_0 + B_1C + B_2I + B_3I*C \quad (1)$$

In this model, p is the percent mortality or teratogenesis observed for MES = *C*, B are the linear coefficients being estimated, I is an indicator function differentiating between the mortality and teratogenicity data (I = 1 for mortality, and I = 0 for teratogenicity), and the logit(p) = ln[p/(1–p)]. The model was constructed as a generalized linear model with a binomial distribution and logit link (MathSoft, 1997). This model is referred to as the full model as opposed to subsequent reduced models.

The first reduced model was designed to test whether the full model better explained the data than a model in which the two dose-responses are identical in terms of both slope and intercept. Consequently the indicator function of the full model was removed, yielding:

$$\text{logit}(p) = B_0 + B_1C \quad (2)$$

The second reduced model was designed to test whether the full model better explained the data than a model in which the intercepts were equal but the slopes differed. This is equivalent to testing for equal slopes and different intercepts in the logit function:

$$\text{logit}(p) = B_0 + (B_1 + B_3)*C \quad (3)$$

The third reduced model was designed to test whether the shapes of the response curves were identical, but shifted:

$$\text{logit}(p) = (B_0 + B_2) + B_1C \quad (4)$$

To compare the full model with the three reduced models, a likelihood ratio test (LRT) was performed for each comparison (Hosmer and Lemeshow, 1989). A significant LRT would indicate that the full model performs significantly better than the reduced model or, in other words, that the slope and/or intercept is statistically significantly different for MES-mortality and MES-teratogenicity exposure-response functions.

RESULTS

Initially, we fit three models (Weibull, logit, and probit) to the pooled data from Heinz *et al.* (1989) and Stanley *et al.* (1994) after the data had been

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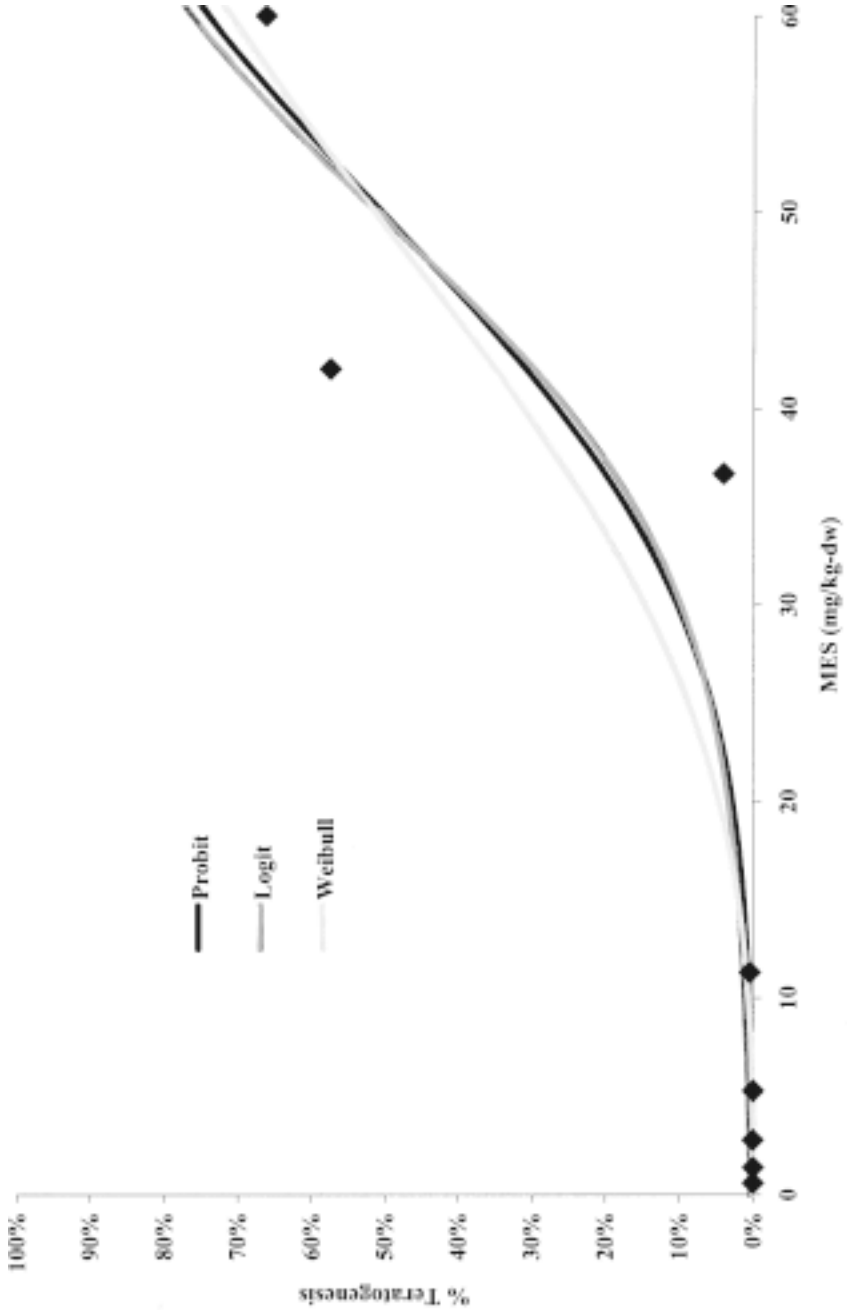


Figure 1. Mean egg selenium (MES) concentration vs. duckling teratogenesis relationship estimated from Heinz *et al.* (1989) and Stanley *et al.* (1994) data, using probit, logit, or Weibull functions. Diamonds indicate reported data points.

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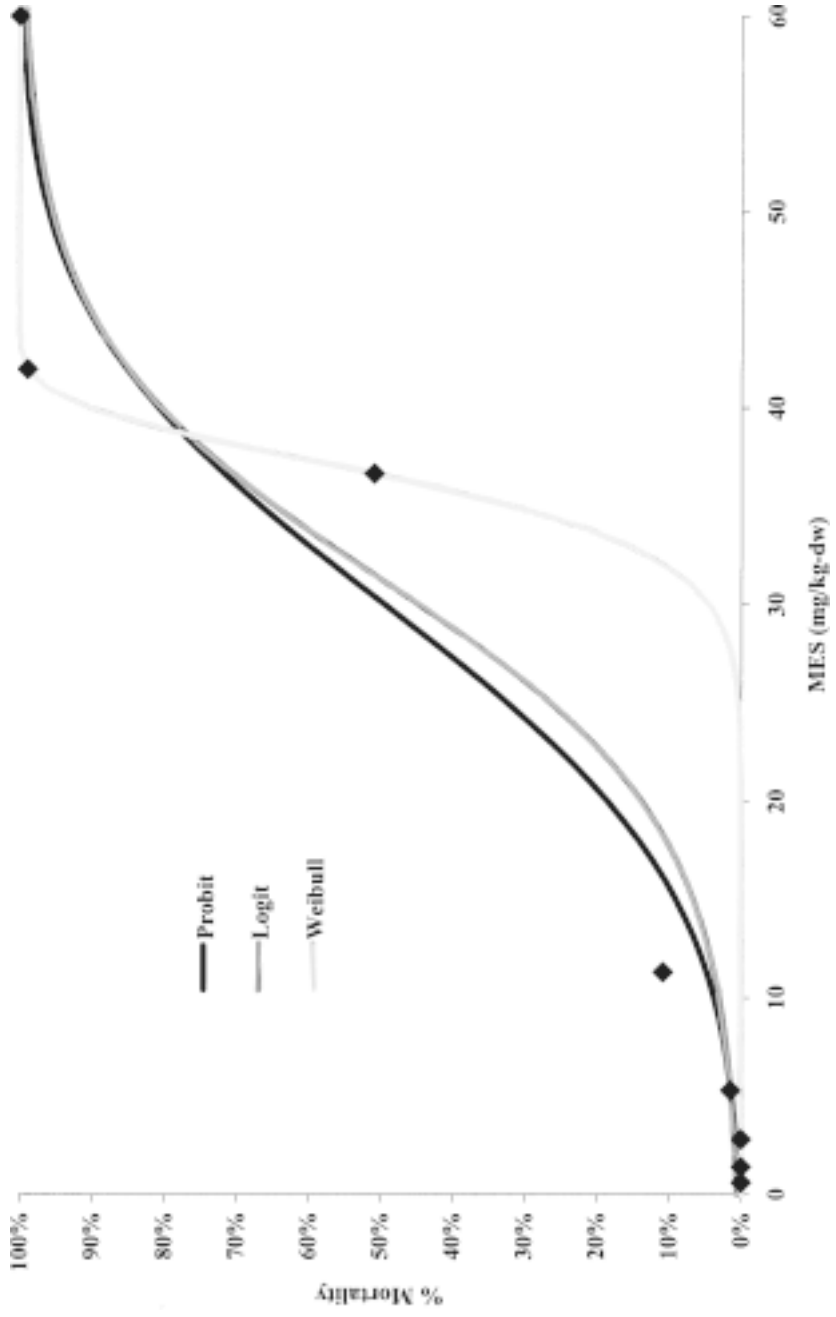


Figure 2. Mean egg selenium (MES) concentration vs. duckling mortality relationship estimated from Heinz *et al.* (1989) and Stanley *et al.* (1994) data, using probit, logit, or Weibull functions. Diamonds indicate reported data points.

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Table 5. Effects thresholds for mortality and teratogenicity endpoints using different models.

Response	Mortality			Teratogenicity		
Model	Probit	Logit	Weibull	Probit	Logit	Weibull
EC10	16	18	32	30	30	26
EC20	21	23	34	37	37	34
EC50	30	31	37	50	50	49

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normalized for control responses from their respective studies. The teratogenicity and mortality endpoints were modeled separately (Figures 1 and 2). The lower tails of the Weibull, logit, and probit models behaved differently, with the Weibull model demonstrating a somewhat different dose-response than the logit and probit models (Figures 1 and 2). For example, the EC_{10} for mortality based on the Weibull model is 32 mg/kg dw MES compared with 16 and 18 mg/kg dw MES for the logit and probit models. While this may seem like a small difference, from a regulatory compliance perspective, the difference between 16 and 32 mg/kg dw MES as an effects threshold is significant. The selection of a threshold level (*e.g.*, EC_{10} vs. EC_{20}) also has implications with EC_{10} values ranging from 16 to 32 and EC_{20} values ranging from 21 to 34 mg/kg dw MES (Table 5).

We further evaluated the sensitivity of the different models and the uncertainty in the data set at 37 and 42 mg/kg dw MES by excluding first the Heinz *et al.* (1989), and then the Stanley *et al.* (1994) data point. This analysis is important because of the uncertainty in this portion of the dose-response curve. Heinz *et al.* (1989) reported 51% mortality and 4% teratogenicity (control normalized) at 37 mg/kg dw MES, while Stanley *et al.* (1994) reported 99% mortality and 53% teratogenicity at only a slightly higher MES concentration of 42 mg/kg dw (Table 4, Figures 1 and 2). These somewhat contradictory results create uncertainty in the dose-response relationships and so it is important to understand how the different models react to this uncertainty.

Results from the sensitivity analysis indicate that when all mortality data are considered, the Weibull model is strongly influenced by the combination of the Heinz (37 mg/kg dw) and the Stanley (42 mg/kg dw) data points, largely ignoring the 11.3 mg/kg dw MES data point from Heinz *et al.* (1989) (Figure 3). Removal of either data point results in the EC_{10} dropping from 32 to 19 or 11 mg/kg dw using the Weibull model. The logit and probit models are not as strongly influenced by individual data points as the Weibull model. Removal of the Stanley data point results in a slight increase in the EC_{10} and removal of the Heinz data point results in a slight decrease in the EC_{10} for both models (Figures 4 and 5).

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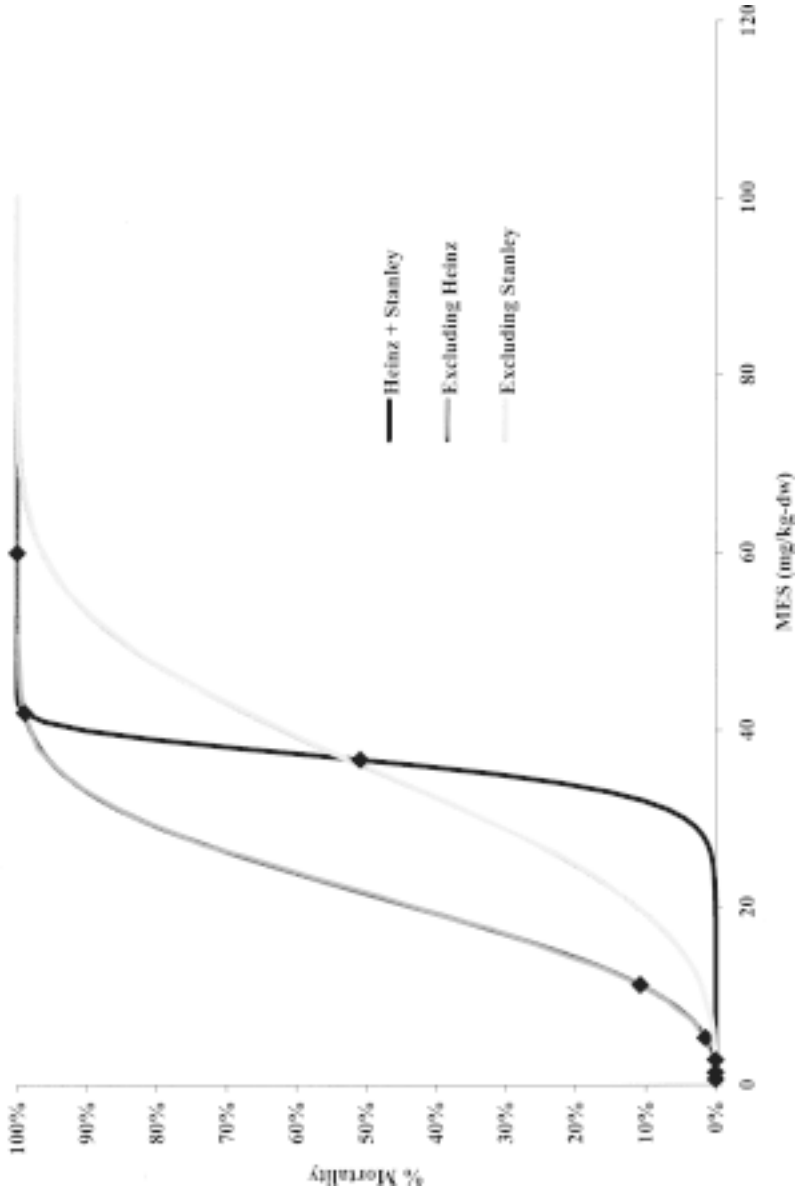


Figure 3. Sensitivity analysis of the influence of including both Heinz *et al.* (1989) and Stanley *et al.* (1994) data at 37 and 42 mg/kg dw, respectively, mean egg selenium (MES) concentration for duckling mortality using the Weibull function to describe the relationship.

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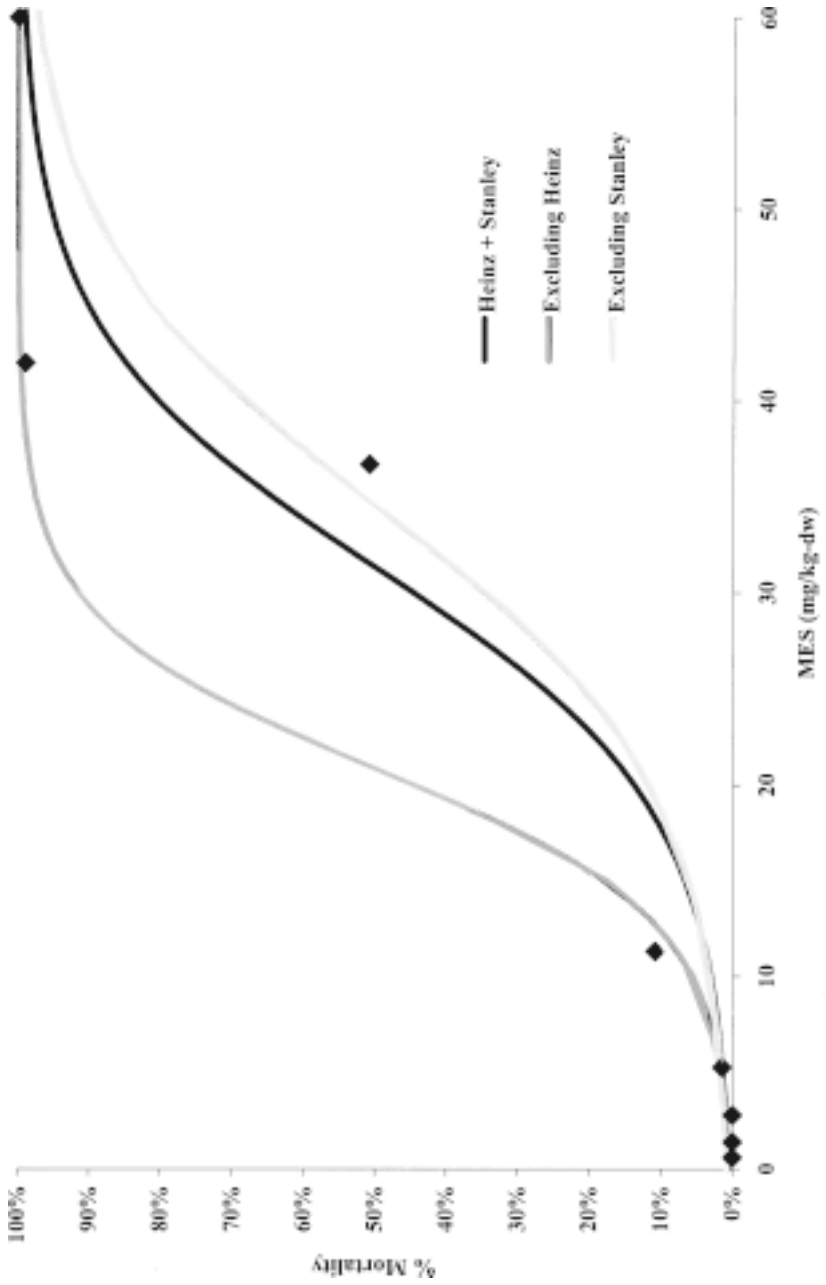


Figure 4. Sensitivity analysis of the influence of including both Heinz *et al.* (1989) and Stanley *et al.* (1994) data at 37 and 42 mg/kg dw, respectively, mean egg selenium (MES) concentration for duckling mortality using the logit function to describe the relationship.

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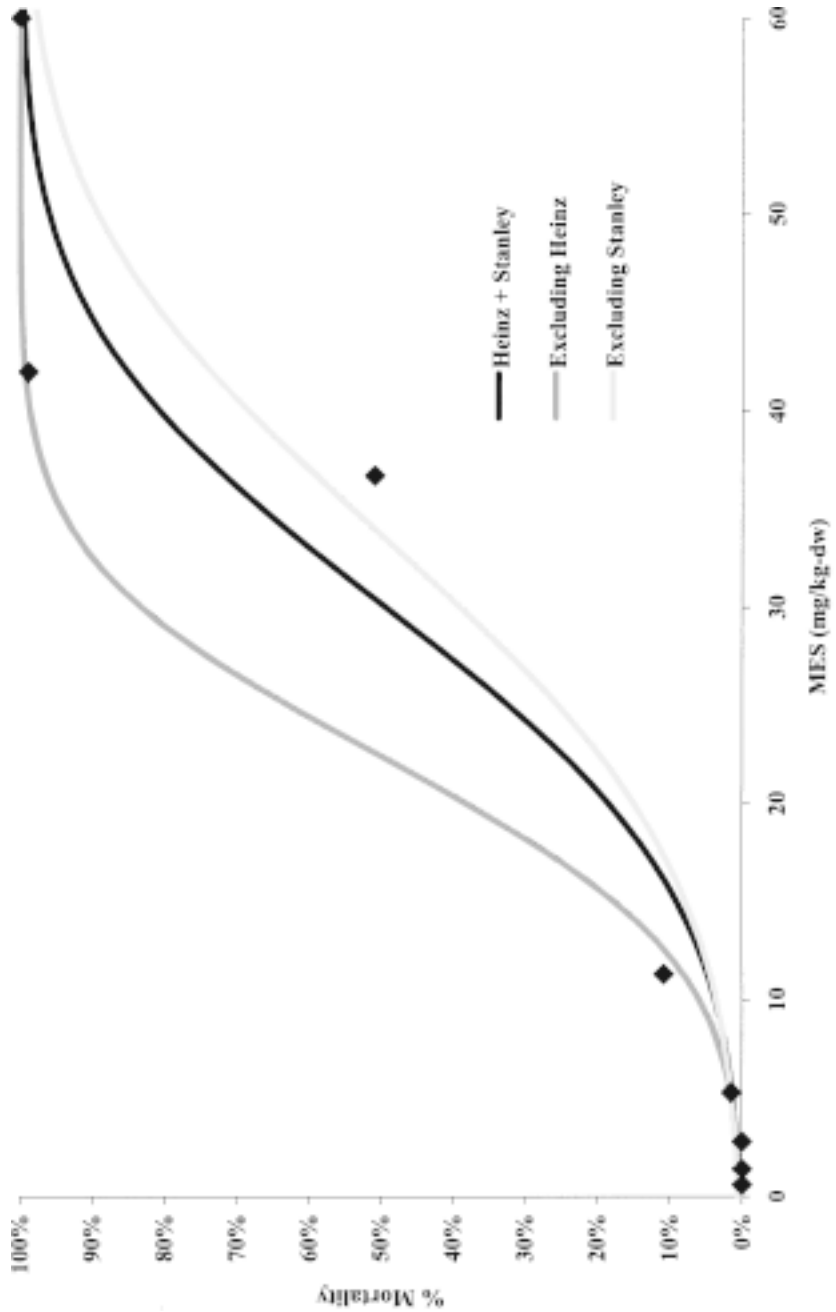


Figure 5. Sensitivity analysis of the influence of including both Heinz *et al.* (1989) and Stanley *et al.* (1994) data at 37 and 42 mg/kg dw, respectively, mean egg selenium (MES) concentration for duckling mortality using the probit function to describe the relationship.

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For the teratogenicity endpoint, all three models are strongly influenced by the uncertainty in the dose-response at the 37 to 42 mg/kg dw MES concentration. Removal of the Stanley data point has the most dramatic effect, raising the teratogenicity EC_{10} from a range of 26 to 30 to a range of 40 to 42 mg/kg dw MES. Removal of the Heinz data point has less of an effect on the logit and probit models, lowering the EC_{10} to 25 mg/kg dw. Removal of the Heinz data point on the Weibull model has a relatively greater effect, lowering the EC_{10} from 26 to 14 mg/kg dw MES.

The results of the sensitivity analyses demonstrate considerable uncertainty in the mortality and teratogenicity dose-response relationships. This uncertainty also influences the evaluation of whether the two endpoints can be distinguished statistically. Results from this analysis indicate that none of the three reduced models significantly differ from the full model (Table 6), that is, the MES-mortality relationship and the MES-teratogenicity relationship cannot be differentiated statistically, based on the shape of the curves, the slopes, or their intercepts. Thus, based on the data in the Heinz *et al.* (1989) and Stanley *et al.* (1994) studies, the mortality endpoint cannot be statistically distinguished as being a more sensitive endpoint than the teratogenicity endpoint.

APPLICATION TO RISK ASSESSMENT

Most wildlife risk assessments are limited in their ability to accurately determine exposure due to the varied and opportunistic diets of most wildlife species. Because dose-response relationships for wildlife are based on feeding studies, an estimate of whether the animal is exposed to chemicals in amounts likely to result in adverse effects is difficult to achieve. Furthermore, risk assessments most frequently are based on single-point toxicity thresholds (*e.g.*, NOAELs or LOAELs) rather than presented in an exposure-response format more representative of a true risk statement. For chemicals such as selenium that accumulate in various body tissues, it is possible to determine more accurately the exposure-response relationship through the use of critical body

Table 6. Results of likelihood ratio tests comparing full model to reduced models.

Model	Likelihood estimate	LRT statistic	*p*-value
Full	0.8293	—	—
No difference	1.2795	0.4503	0.798
Equal intercepts, differing slopes	0.8296	0.0003	0.986
Equal slopes, differing intercepts	0.8740	0.0448	0.832

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residues. However, we urge that "natural background" tissue concentrations (including egg concentrations) not be used for risk assessment or criteria setting. There are significant difficulties in determining what constitutes "natural" exposures, particularly for an element such as selenium that has large spatial variations in soil concentrations. Additionally, exceedance of background concentrations does not equate to effects and consequently does not equate to risk.

Selenium is a particularly data-rich chemical from the standpoint of risk assessment. Because of the field observations and laboratory studies conducted in the last 15 years, we have information on toxic responses as a result of dietary exposure. Our reanalysis of these studies has demonstrated that excess duckling teratogenicity and mortality are statistically equally sensitive reproductive endpoints given the extant laboratory data.

Despite being unable to statistically distinguish between the two endpoints, development of dose-response relationships does suggest that mortality is a more sensitive endpoint than teratogenicity. However, it is important to point out that the Heinz *et al.* (1989) and Stanley *et al.* (1994) data indicate that the mortality endpoint is only 1.7 to 1.9 times more sensitive than the teratogenicity endpoint depending on the model used. This differs significantly from the relationship indicated by Skorupa *et al.* (1996) and Skorupa (1998). These authors developed field-based thresholds of 6 and 20 mg/kg dw MES for mortality and teratogenicity, respectively, indicating mortality is 3.3 times more sensitive than teratogenesis. The laboratory studies were performed under controlled conditions where mortality only associated with selenium could be evaluated while the field mortality data were subject to numerous confounding factors such as weather, predators, flooding, disease and other contaminants. Further, the field-based mortality threshold is 2.6 times lower than the lab-based threshold while the field-based teratogenicity threshold is only 1.5 times lower than the lab-based threshold. This would all tend to indicate that the field-based mortality threshold of 6 mg/kg dw is confounded by other factors and should not be used for regulatory purposes until it undergoes extensive external peer review.

Considering the above, the laboratory data appear to be the most appropriate for developing regulatory thresholds. We have constructed relationships between the mortality and teratogenicity endpoints and MES, an easily measured exposure indicator. These relationships provide the risk manager with an estimate of the probability of teratogenicity or mortality if MES is known. Thus, the desired level of protection can be adjusted depending on the degree of risk aversion of the manager and associated decision makers, rather than being set *a priori*. Depending on which model is used, the EC_{10} for mortality is between 16 and 32 mg/kg dw MES. As demonstrated in the sensitivity analysis, the Weibull model (EC_{10} of 32 mg/kg dw) appears unduly influenced by the uncertainty in the dose-response, while the logit and probit models are largely in agreement. Consequently, we recommend the more conservative of these two models, namely 16 and 21 mg/kg dw MES based on the logit model,

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as the best estimates of the mortality EC_{10} and EC_{20}. Similarly, for the teratogenicity endpoint, we recommend use of the logit model with resulting EC_{10} and EC_{20} values of 30 and 37 mg/kg dw MES. The EC_{10} is higher (30 vs. 20 mg/kg dw) than that estimated by Skorupa *et al.* (1996) based on field teratogenicity data for mallards.

While we are fortunate to have data on selenium effects in mallards that were collected in a rigorous, scientifically defensible manner and presented in the peer-reviewed literature, the information pertaining to relative sensitivity of other wildlife species has not been as well documented. It appears from summary reports of field data that mallards may be fairly sensitive, with some species (*e.g.*, stilts and avocets) less sensitive and others perhaps slightly more sensitive (*e.g.*, coots) to selenium-induced teratogenesis and potentially to mortality if the latter proves to be a more sensitive endpoint. Information is lacking about how the data on egg selenium concentrations and teratogenic levels in species other than mallards were collected; hence, the dose-response relationships for these species will continue to carry a large uncertainty. We believe such information should be used only to estimate relative species differences, as we have done here, and not for setting stringent regulatory standards. Considering this, we believe the uncertainty in the current dose-response data and the relative sensitivity between species could be reduced by conducting two additional laboratory studies (one on mallards and the other on coots). These studies should include a large number of test concentrations (*e.g.*, 10) in the lower range of the dose-response in order to accurately estimate the EC_{10} and EC_{20}, the effect thresholds most commonly used in a regulatory context.

Finally, it is important to recognize that the teratogenicity and mortality endpoints discussed here relate selenium exposure to risk of individuals within a population. They do not provide information about the probability of selenium causing changes in population growth rates, either at the local or regional levels. An assessment of selenium risks to the continued maintenance of a bird population can only be done if information is available on the demographics of the species of interest, such as birth and death rates, or on other estimates of annual recruitment rates. Influences of climate, predation and other environmental stressors would also need to be integrated into such an assessment. However, the exposure-response function does express the contribution of selenium exposure to reproductive recruitment success in a manner that can be integrated easily into population models.

In conclusion, we believe that sufficient data exist to develop protective and predictive toxicity response information for selenium exposure of individual birds. This is a tissue based approach that reduces the uncertainty associated with predictions based on concentrations in water or dietary items. Once full detail of the field data on teratogenicity responses in stilts, avocets, and other water birds are available, it may be possible to make predictions for multiple species. Until then, although uncertainty remains, the response functions presented in this paper provide a model on which to base environmental management decisions.

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