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Selenium in Birds

6

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7

and

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Introduction

10 Selenium (Se) is a metalloid trace element that birds and other wildlife need in small
11 amounts for good health. The main purpose of this chapter is to interpret tissue
12 concentrations of Se. However, because food is the main source of Se accumulation
13 for birds and other wildlife, and because dietary concentrations for effects on bird
14 reproduction have been reported, we also provide interpretive information on Se in
15 the diet.

16 Selenium deficiencies in domestic poultry and livestock occur in some parts of the
17 world and must be corrected by additions of Se to the diet. However, the range of
18 dietary concentrations that provides adequate but nontoxic amounts of Se is narrow
19 compared with the ranges for most other essential trace elements.

20 In the 1930s, grains grown on seleniferous soils in South Dakota caused
21 reproductive failure when fed to chickens (*Gallus domesticus*) (Poley and Moxon,
22 1938). The most drastic incident of Se poisoning in wild birds occurred at Kesterson
23 Reservoir (located on the Kesterson National Wildlife Refuge) in California during
24 the early and mid-1980s (Ohlendorf et al., 1986a, 1988; Ohlendorf and Hothem, 1995;
25 Ohlendorf, 1989, 2002). Water used to irrigate crops in the San Joaquin Valley of
26 California dissolved naturally occurring Se salts from the soil, and when the Se-
27 laden subsurface water was drained from agricultural fields into Kesterson
28 Reservoir, levels of Se that were toxic to birds accumulated in plants and animals
29 used as foods by the birds. Reproductive failure and adult mortality occurred. The

30 findings at Kesterson Reservoir received extensive publicity and led to a series of
31 laboratory and field studies (summarized in this chapter) that provide one of the
32 best case studies in ecotoxicology during the past 30 years. The integrated field
33 studies at Kesterson and related laboratory studies have been recognized as a “gold
34 standard” in the field of ecotoxicology (Suter, 1993). Similar problems of impaired
35 bird reproduction were subsequently discovered elsewhere in the western United
36 States, most notably in the Tulare Basin in California (Skorupa and Ohlendorf, 1991;
37 Skorupa, 1998a).

38 High concentrations of Se in foods of wildlife are not limited to areas where soils are
39 naturally high in Se. They also can be the result of the disposal of sewage sludge or
40 fly ash, mining activity, or emissions from metal smelters (Robberecht et al., 1983;
41 Wadge and Hutton, 1986; Cappon, 1991; Skorupa, 1998a; Ratti et al., 2006; Wayland
42 and Crosley, 2006).

43 An assessment of the toxicity of Se is complicated by its occurrence in many
44 different chemical forms, some differing greatly in their toxicity to birds. The four
45 common oxidation states are selenide (-2), elemental Se (0), selenite (+4), and
46 selenate (+6). Elemental Se is virtually insoluble in water and presents little risk to
47 birds. Both selenite and selenate are toxic to birds, but organic selenides pose the
48 greatest hazard. Among the organic selenides, selenomethionine has been shown to
49 be highly toxic to birds and seems to be the form most likely to harm wild birds
50 because it results in high bioaccumulation of Se in their eggs.

51 Much has been learned about Se toxicity to birds during the last 25 years; some of
52 that information was summarized in the earlier edition by Heinz (1996). Other
53 reviews in relation to exposure and effects of Se in birds are provided by Skorupa
54 (1998a), O’Toole and Raisbeck (1998), USDI (1998), Eisler (2000), Hoffman (2002),
55 and Ohlendorf (2003). The purpose of this chapter is to identify the concentrations of
56 Se in avian diets and in avian eggs and other tissues that are toxic, and to discuss
57 how different chemical forms of Se and their interactions with other environmental

58 contaminants can alter toxicity. We also present what are considered background (or
59 no-effect) concentrations of Se from Se-normal areas, when available.

60 Background and reference area concentrations can be very useful for interpreting
61 the possible toxic thresholds of a contaminant, especially when it is known with
62 some certainty that the reference area has no known source of the contaminant in
63 question. However, because some 'background' concentrations of contaminants
64 such as Se are reported from areas where the Se input is unknown, and may not, in
65 fact, be what might be called 'normal,' 'baseline,' or 'uncontaminated,' they should
66 be referred to as 'reference area' samples, and a certain degree of caution must be
67 exercised when using those concentrations as being synonymous with safe levels.
68 The rigorous identification of safe levels of Se, or other contaminants, can really
69 come only from the findings of controlled laboratory dosing studies and carefully
70 designed field studies. In other words, merely because a contaminant like Se is at a
71 level that has been reported from what are believed to be Se-normal areas does not,
72 in itself, prove that the levels are safe.

73 The manner in which different authors present Se concentrations can be confusing,
74 so it is important to understand the various ways results can be presented. Selenium
75 concentrations typically are reported as micrograms per liter ($\mu\text{g}/\text{L}$) in most fluids
76 (but sometimes $\mu\text{g}/\text{g}$ or $\mu\text{g}/\text{dL}$ in blood) and milligrams per kilogram (mg/kg) or
77 micrograms per gram ($\mu\text{g}/\text{g}$) in soil, sediment, plant or animal tissues, and diets.
78 Concentrations in soil, sediment, tissues, and diets can be expressed either on a wet-
79 weight (or fresh-weight basis, which is considered to be synonymous) or a dry-
80 weight basis. Although moisture loss during sample processing can be controlled
81 fairly well in the laboratory, it is sometimes difficult to do so under field conditions.
82 Therefore, reporting results on dry-weight basis helps ensure comparability of
83 values.

84 Conversion from one basis to the other is a function of the moisture content in the
85 sample (which should be reported regardless of which basis is used), as follows:

86 Dry-weight conc. = wet-weight conc. X 100/(100 - percentage moisture)
87

88 In this chapter, we preferentially provide Se concentrations in diets and tissues on
89 dry-weight (dw) basis (unless otherwise noted), and provide typical moisture
90 content of eggs and tissues to enable readers to make conversions. When results
91 were originally reported on wet-weight (ww) basis, the original concentrations are
92 given in parentheses following the approximate dw concentration.

93 Selenium's ability to interact with other nutrients and environmental contaminants,
94 especially other elements, also sometimes complicates an interpretation of toxic
95 thresholds in tissues of birds. Although we do not attempt a comprehensive review
96 to interpret critical levels of Se in the presence of elevated levels of other pollutants,
97 we include a brief section on interactions, and the reader should be aware that such
98 interactions exist.

99 **Dietary Requirements versus Toxicity**

100 In general, the diet is the most important exposure pathway for birds and, whenever
101 possible, dietary concentrations should be included when reporting results or
102 evaluating the effects observed in experimental or field studies. With the previously
103 stated caution about 'background' levels of Se in mind, mean background
104 concentrations in diets of freshwater and terrestrial avian species are typically < 3
105 mg/kg, with thresholds for reproductive impairment in the range of 3 to 8 mg/kg
106 (Table 1).

107 For birds, as for most other animals, dietary Se requirements appear to be between
108 about 0.05 and 0.5 mg/kg (NAS-NRC, 1976, 1983; Combs and Combs, 1986;
109 Oldfield, 1990, 1998; Eisler, 2000). Excess Se in the diet of female birds during the
110 period just before egg-laying can result in the transfer of Se to the eggs or other
111 tissues at harmful levels, although sensitivity to Se varies among species (Ohlendorf,
112 1996; Skorupa, 1998a, b; Skorupa and Ohlendorf, 1991). Detwiler (2002) analyzed
113 field-collected eggs and conducted laboratory studies with chickens to determine

114 partitioning of Se in eggs (to albumen, yolk, and embryo) and to identify
115 toxicokinetic causes of species variability in sensitivity to Se. As expected,
116 differences among species, as well as those due to form of Se in the diet, are
117 complex. Those complexities are not described in detail here, but readers may wish
118 to read about them in Detwiler's (2002) work.

119 Ohlendorf (2003) used data from six laboratory studies with mallards (*Anas*
120 *platyrhynchos*) (Heinz et al., 1987, 1989; Heinz and Hoffman, 1996, 1998; Stanley et al.,
121 1994, 1996) to calculate an EC₁₀ (i.e., the 'effective concentration' that caused a 10%
122 effect; in this case, the dietary concentration that reduced hatching of eggs 10%
123 below that of the control group in the same study) along with 95% confidence
124 intervals (95% CI) for the mean Se concentration in the diet. The dietary EC₁₀ was
125 calculated to be 4.9 mg Se/kg, with 95% CI of 3.6 to 5.7 mg Se/kg.

126 The EC₁₀ of 4.9 mg Se/kg was estimated by fitting a logistic regression model to the
127 available data. It should be noted, however, that the mallard studies used a "dry"
128 diet that had about 10% moisture. Ohlendorf (2003) used the reported dietary Se
129 concentrations without adjustment for that moisture content, but an upward
130 adjustment of the values (by 11%; to about 5.4 mg/kg) would be appropriate to
131 account for the moisture content of the duck diet.

132 Adams et al. (2003) used hockey-stick regression on data for egg Se concentrations
133 and adverse effects in mallards to derive toxicity thresholds, such as EC₁₀ values.
134 Upon further analyses (as described in Ohlendorf, 2007), they found a threshold to
135 exist when dietary Se was plotted against egg inviability and duckling mortality
136 (which incorporated the cumulative effects of fertilization success and hatchability
137 plus survival of ducklings for 6, 7, or 14 days after hatching, as reported for the
138 different studies). The inflection point occurred at a dietary Se concentration of 3.9
139 mg/kg. The predicted EC₁₀ was 4.4 mg Se/kg (just slightly above the inflection
140 point) and the 95% CI around the predicted EC₁₀ ranged from 3.8 to 4.8 mg Se/kg.

141 Wayland et al. (2007) used logistic regression to calculate EC₁₀ values based on
142 experimental studies of six species (mallard, American kestrel [*Falco sparverius*],
143 domestic chicken, black-crowned night-heron [*Nycticorax nycticorax*], eastern
144 screech-owl [*Megascops asio*] and ring-necked pheasant [*Phasianus colchicus*]). The
145 EC₁₀ was 4.0 mg Se/kg with 95% CI from <0.5 to 7.3 mg Se/kg. The effect of
146 including several species was to widen the confidence limits substantially
147 (compared to mallard EC₁₀), indicating a high degree of difference among species in
148 sensitivity to Se.

149 Information on forms of Se in invertebrates (as potential diets for birds) is limited,
150 but Andrahennadi et al. (2007) found variability in the Se speciation among aquatic
151 insects that included mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies
152 (Trichoptera), and craneflies (Diptera) from streams in Alberta, Canada. Higher
153 percentages of inorganic Se were found in primary consumers, detritivores, and
154 filter feeders than in predatory insects. Among the organic forms, organic selenides
155 constituted a major fraction in most organisms. A form of selenide, believed to
156 represent selenomethionine, varied widely among aquatic insects (from 36-98% of
157 the total Se), indicating a high degree of variability in bioaccumulation potential
158 from diet to eggs. Nevertheless, the chemical forms of Se in aquatic foods of birds
159 have received little study. It is likely that varying chemical forms of Se are present to
160 some degree in plants and animals eaten by birds, yet the toxic concentrations of few
161 Se compounds have been determined in birds.

162 Interpretive guidelines that have resulted from extensive testing with poultry are
163 provided by Puls (1988). The Se concentrations for diet (as well as those for eggs and
164 other tissues) are helpful guidelines for wild birds as well as domestic poultry.

165 Dietary Se concentrations of less than 0.30 mg/kg are considered to be below the
166 range adequate for good adult health and reproduction, 3.0 to 5.0 mg/kg are high,
167 and above 5.0 mg/kg are toxic (Table 1).

168 **Egg and Tissue Concentrations**

169 Eggs

170 Mean background Se concentrations in eggs of freshwater and terrestrial birds are <
171 3 mg/kg dw (typically 1.5-2.5 mg/kg dw; concentrations lower than about
172 0.66 mg/kg dw may indicate inadequate Se in the diet, and maximums for
173 individual eggs are <5 mg/kg dw (Table 1). Moisture content of eggs varies by stage
174 of incubation (decreasing throughout incubation) and by species, but typical
175 moisture content of field-collected eggs is usually 65 to 80% (Ohlendorf and
176 Hothem, 1995). Fresh mallard eggs, such as those collected from laboratory studies,
177 have about 70% moisture (Stanley et al., 1996). The latter value provides a
178 reasonable conversion factor (3.3) for estimating from one basis to the other and,
179 except where noted, is used in this chapter when Se concentrations in eggs were
180 originally reported on wet-weight basis, but the moisture content of samples was
181 not reported.

182 Laboratory Studies

183 In a wide variety of species, if one expresses both the diet and eggs on a dry-weight
184 basis, Se concentrations in bird eggs range from roughly equal to about three or four
185 times the concentrations in the diet of the female at the time of egg-laying (Heinz et
186 al., 1987, 1989; Smith et al., 1988; Ohlendorf, 1989; Stanley et al., 1994, 1996;
187 Wiemeyer and Hoffman, 1996; Santolo et al., 1999). However, Se transfer from diet
188 to egg varies by species and the chemical form of Se in the diet.

189 When birds fed on Se-contaminated diets during the laying season, the exposure
190 was quickly reflected in elevated levels of Se in eggs (Heinz, 1993b; Latshaw et al.,
191 2004; DeVink et al., 2008a). Similarly, when the birds were switched to a clean diet,
192 Se concentrations in eggs declined quickly. When mallard hens were fed a diet
193 containing 15 mg Se/kg (as selenomethionine), levels peaked in eggs (to about 43 to
194 66 mg Se/kg dw; 13-20 mg Se/kg ww) after about 2 weeks on the treated diet and
195 leveled off at a relatively low level (<16 mg Se/kg dw; <5 mg Se/kg ww) about 10
196 days after switching to an untreated diet (Heinz, 1993b). The findings of this study

197 and two others with ring-necked pheasants (*Phasianus colchicus*) (Latshaw et al.,
198 2004) or lesser scaup (*Aythya affinis*) (DeVink et al., 2008a) summarized below have
199 important implications for evaluation of field exposures, such as how quickly and
200 for what duration Se exposure may adversely affect bird reproduction.
201 Concentrations of Se in eggs are especially important because they provide the best
202 samples for evaluating potential adverse reproductive effects (Skorupa and
203 Ohlendorf, 1991). Knowing Se concentrations in food items available to wild birds at
204 a site also can be useful in assessing risks of reproductive effects, but relationships
205 between the available food and concentrations that occur in eggs can vary widely on
206 the basis of physiology and feeding ecology of the birds. Selenium speciation in the
207 diet also may be important in this regard (i.e., plant versus animal diets).

208 When ring-necked pheasants received feed that contained 9.3 mg Se/kg because of a
209 feed mixing problem, severe effects occurred within 4 days (Latshaw et al., 2004).
210 The rate of egg production decreased and bird aggression increased. About 12% of
211 the hens died within a week; necropsy results were consistent with Se toxicity. After
212 8 days, the toxic feed was removed and replaced with fresh feed. Egg production,
213 which had dropped by 50%, returned to normal within 10 days of feed replacement.
214 Hatchability of eggs laid from days 8 to 14 after the pheasants received the toxic feed
215 dropped to 35%, and more than 50% of the embryos that survived to the point
216 where they could be examined had deformed beaks and abnormal eyes. Hatchability
217 of eggs laid 21 to 28 days after the hens had received the toxic feed (i.e., 13 to 20 days
218 after it was replaced by new feed) was almost 80%. Similar to the study with
219 mallards, this incident showed a rapid onset of effects and a rapid recovery in
220 response to dietary Se concentrations.

221 To assess the possible effects of Se on reproduction and fitness (measured as body
222 mass) of lesser scaup, captive scaup were fed a control diet or one supplemented
223 with Se at 7.5 or 15 mg/kg for 30 days to simulate dietary exposure to Se during late
224 spring migration (DeVink et al., 2008a). The treated feed was removed after 30 days,

225 just before the birds began laying. There was no effect of Se on body mass, breeding
226 probability, or clutch initiation dates. Selenium concentrations in the first eggs laid
227 by these birds were 25 to 30 mg/kg in the 7.5-mg/kg and 30 to 35 mg/kg in the 15-
228 mg/kg treatment groups. Egg Se concentrations of both treatment groups decreased
229 rapidly after the Se-supplemented feed was removed, and within 8 days and 12
230 days, respectively, the egg Se concentration was less than 9 mg/kg dw. There was
231 no significant intraclutch variation in egg Se deposition.

232 The embryo is the avian life stage most sensitive to Se (Poley et al., 1937; Poley and
233 Moxon, 1938; Heinz et al., 1987, 1989; Hoffman and Heinz, 1988). Because it is the Se
234 in the egg, rather than in the parent bird, that causes developmental abnormalities
235 and death of avian embryos, Se in the egg gives the most sensitive measure for
236 evaluating hazards to birds (Skorupa and Ohlendorf, 1991). Given the rapid
237 accumulation and loss patterns of Se in birds (Heinz et al., 1990; Heinz, 1993b; Heinz
238 and Fitzgerald, 1993b; Latshaw et al., 2004), Se concentrations in eggs also probably
239 best represent contamination of the local environment. Additional advantages of
240 measuring Se in eggs are that eggs are frequently easier to collect than adult birds,
241 the loss of one egg from a nest probably has little effect on a population, and the egg
242 represents an integration of exposure of the adult female during the few days or
243 weeks before egg-laying.

244 The concentration detected in eggs and the toxicity of that concentration seem to
245 depend on the chemical form of the ingested Se. Organoselenium compounds are
246 believed to be major forms in plants and animals. One organoselenium compound,
247 selenomethionine, when fed to breeding mallards was more toxic to embryos than
248 was selenocystine or sodium selenite (Heinz et al., 1989). Selenomethionine is a
249 major form of Se in wheat seeds and soybean protein (Olson et al., 1970; Yasumoto
250 et al., 1988). Hamilton et al. (1990) found selenomethionine to be an excellent model
251 for Se poisoning in Chinook salmon (*Oncorhynchus tshawytscha*) when compared
252 with the toxicity of Se that was biologically incorporated into mosquitofish

253 (*Gambusia affinis*) collected at Kesterson Reservoir in California. Yamamoto et al.
254 (1998) measured Se concentrations in blood and excreta of American kestrels fed
255 either a selenomethionine-fortified diet or animals from Kesterson. They found no
256 significant differences in concentrations or in accumulation and depuration of Se
257 among experimental groups that received Se as selenomethionine or naturally
258 incorporated in tissue of animals from Kesterson.

259 When mallards were fed a diet containing 10 mg Se/kg as selenomethionine (and
260 about 10% moisture), reproductive success was significantly lower in the treated
261 ducks than in controls, and a small sample of five eggs from the treated birds
262 contained a mean of about 15 mg Se/kg dw (4.6 mg Se/kg ww) (Heinz et al., 1987).
263 Because mallards were fed only one dietary concentration of Se in the form of
264 selenomethionine, no safe level was established in this experiment. All that can be
265 said is that the safe level in eggs was below about 15 mg Se/kg dw.

266 In a subsequent study, mallards were fed a diet containing about 10% moisture and
267 0, 1, 2, 4, 8, or 16 mg/kg of added Se as selenomethionine (Heinz et al., 1989). The
268 reproductive success of the groups fed 1, 2, or 4 mg Se/kg did not significantly
269 differ from that of controls; mean Se concentrations in a sample of 15 eggs from each
270 of these groups were about 2.7, 5.3, and 11 mg/kg dw (0.83, 1.6, and 3.4 mg/kg ww).
271 The group fed 8 mg Se/kg produced 57% as many healthy ducklings as the controls;
272 the reduction in numbers was caused mainly by hatching failure and the early death
273 of those that did hatch. A sample of 15 eggs from this group contained about 36 mg
274 Se/kg dw (11 mg Se/kg ww). The group fed 16 mg Se/kg failed to produce any
275 healthy young, and a sample of 10 of their eggs contained an average of about 59 mg
276 Se/kg dw (18 mg Se/kg ww). Therefore, based on this study, the highest mean Se
277 concentration in eggs not associated with reproductive impairment was about 11
278 mg/kg dw (3.4 mg/kg ww), and the lowest mean toxic concentration was 36 mg/kg
279 dw (11 mg/kg ww).

280 Lam et al. (2005) subjected the data from this study with mallards (Heinz et al., 1989)
281 to statistical analyses to estimate the threshold for effects on clutch viability. They
282 normalized treatment response for control response and subjected the data to linear
283 regression analysis, and then used a stepwise increment of 0.5-mg Se/kg
284 concentration units followed by a one-tailed, one-sample t-test comparing the
285 percentage of impairment of clutch viability ($\pm 95\%$ CI) with zero to derive threshold
286 effect levels of Se in eggs associated with impaired hatchability. They determined
287 that 9 mg Se/kg was the lowest concentration in eggs at which clutch viability was
288 significantly different than zero, and that the value represented an $EC_{8.2}$ for effects.
289 A recent paper by Beckon et al. (2008) used the mean response data from the same
290 laboratory study with mallards (Heinz et al., 1989) to evaluate potential hormetic
291 effects exhibited by the treatment groups, and found an EC_{10} of 7.7 mg Se/kg (see
292 later section on Hormesis).

293 In another study, Heinz and Hoffman (1996) compared the toxicity of three forms of
294 selenomethionine. In nature, selenomethionine occurs almost exclusively in the L
295 form, which is one of the two stereoisomer forms it can take (Cukierski et al., 1989).
296 The other stereoisomer is the D form, and in many feeding studies with birds a
297 mixture of the two forms (seleno-DL-methionine) has been fed. In yeast, most of the
298 Se is in the form of seleno-L-methionine (Beilstein and Whanger, 1986), and in
299 addition to being in the naturally-occurring form, it is biologically incorporated into
300 the yeast. Pairs of breeding mallards were fed 10 mg Se/kg in each of the three
301 forms. The results suggested that seleno-DL-methionine and seleno-L-methionine
302 were of similar toxicity and both were more toxic than the Se in selenized yeast, but
303 the lower toxicity of selenized yeast may have been due to a lower bioavailability of
304 the selenomethionine in the yeast. A sample of eggs from the pairs fed seleno-L-
305 methionine contained a mean of about 30 mg Se/kg dw (8.9 mg Se/kg ww), which
306 resulted in a severe reduction in reproductive success (6.4% hatching of fertile eggs
307 compared to 41.3% for controls). Eggs from pairs fed the seleno-DL-methionine
308 contained a mean of about 31 mg Se/kg dw (9.2 mg Se/kg ww), and hatching of

309 fertile eggs was 7.6%. Eggs from the pairs fed the selenized yeast contained a mean
310 of only about 22 mg Se/kg dw (6.6 mg Se/kg ww), and hatching success was 27.0%.
311 Because even the 22 mg Se/kg derived from the selenized yeast had a profound
312 effect on reproductive success a toxic threshold was not established, but was
313 obviously well below 22 mg Se/kg. Three studies were conducted to evaluate the
314 interactive effects of Se with arsenic (As) (Stanley et al., 1994), boron (B) (Stanley et
315 al., 1996), or mercury (Hg) (Heinz and Hoffman, 1998), which are described in a later
316 section (Interactions).

317 Using the same approach as that described above for the dietary values associated
318 with reduced egg hatchability in mallards, Ohlendorf (2003) found the EC₁₀ in eggs
319 was 12 mg Se/kg dw, with 95% CIs of 6.4 to 16 mg Se/kg dw. The EC₁₀ of 12 mg
320 Se/kg was estimated by fitting a logistic regression model to the results of the six
321 laboratory studies with mallards mentioned above.

322 The EC₁₀ for mallard duckling mortality, as reported in Adams et al. (2003), ranged
323 from 12 to 16 mg Se/kg dw in eggs. These EC₁₀ values are based on a synthesis of
324 the same six laboratory studies as above, but using the final endpoint of duckling
325 mortality (the same effects data used in the dietary EC₁₀ evaluation with hockey-
326 stick regression above); the range of EC₁₀ values reflects different statistical
327 approaches for analyzing the data. Based on further analyses of those data, Adams
328 (pers. comm.; see Ohlendorf, 2007]) determined that the inflection point of the
329 hockey stick occurred at an egg Se concentration of 9.8 mg/kg dw, with a predicted
330 EC₁₀ of about 12 mg/kg dw, which was comparable to that derived by Ohlendorf
331 (2003). The 95% CI using hockey-stick regression was much narrower (9.7 to
332 14 mg/kg dw) than that derived by Ohlendorf using logistic regression (6.4 to
333 16 mg/kg dw). Given that there is a clear egg-Se threshold at which effects begin to
334 be observed, a unimodal model, such as logistic regression, may result in
335 exaggerated confidence intervals, particularly in the tails.

336 In a laboratory study designed to measure the lingering effects of an overwinter
337 exposure to selenomethionine on reproduction, mallards were fed a diet containing
338 15 mg Se/kg for 21 weeks before the onset of laying (Heinz and Fitzgerald, 1993b).
339 Females began laying after various lengths of time off treatment. This experimental
340 design was not ideal for determining the lowest concentration of Se in eggs
341 associated with reproductive impairment, but the authors were able to make some
342 general conclusions. Some eggs hatched when Se in eggs was as high as about 20 to
343 30 mg/kg dw (6 to 9 mg/kg ww), but other eggs failed to hatch when Se
344 concentrations were estimated to be between 9.9 and 16 mg/kg dw (3 and 5 mg/kg
345 ww). The authors concluded that the most logical reason why some embryos die
346 while others survive when exposed to a given concentration of Se is that mallard
347 embryos vary in their individual sensitivity to Se.

348 When black-crowned night-herons were fed a diet containing 10 mg Se/kg as
349 selenomethionine (on close to a dry-weight basis) hatching success of fertile eggs
350 was not reduced (Smith et al., 1988). The eggs of treated herons contained a mean
351 concentration of about 11 mg Se/kg dw (3.3 mg Se/kg ww). The results from this
352 study must be taken with some caution, however, because sample sizes were small
353 (n = 5 pairs per group) and hatching success of fertile eggs of the control group was
354 poor (32%).

355 Martin (1988) fed Japanese quail (*Coturnix coturnix japonica*) diets containing 5 or 8
356 mg Se/kg and chickens 10 mg Se/kg as selenomethionine, respectively. At 5 mg
357 Se/kg, the hatching success of fertile quail eggs (56.4%) was lower than that of
358 controls (76.4%); eggs from treated females contained about 23 mg Se/kg dw (7.1
359 mg Se/kg ww). At 8 mg Se/kg, the hatching of quail eggs was further decreased to
360 10.4% (compared with 75.1% for controls in that trial), and Se in eggs averaged
361 about 40 mg/kg dw (12 mg/kg ww). The hatching success of the chickens fed 10 mg
362 Se/kg also was depressed (23.2% compared with 84.5% for controls), and Se in eggs
363 averaged about 36 mg/kg dw (9.6 mg/kg ww; the conversion from ww to dw [3.8]

364 was based on the contents of chicken eggs containing about 73.6% water [Romanoff
365 and Romanoff, 1949)]. No-effect concentrations in the diet or eggs were not
366 determined.

367 In another study with chickens, diets were supplemented with seleniferous grains in
368 amounts to produce dietary concentrations of 2.5, 5, and 10 mg Se/kg (Poley and
369 Moxon, 1938; Moxon and Poley, 1938). Modern statistical techniques were not
370 applied to these data, and chemical analyses were different from those used today,
371 but at 2.5 mg Se/kg in the diet, the hatching success of fertile eggs was no different
372 from that of controls, and a sample of eggs contained Se at about 15 mg/kg dw in
373 albumen and 3.2 mg/kg dw in yolk (1.75 mg/kg and 1.67 mg/kg ww, respectively;
374 conversions from ww to dw here and below [multiply ww concentrations by 8.3 for
375 albumen and by 1.9 for yolk] were based on the fact that chicken eggs are composed
376 of about 55.8% albumen, 31.9% yolk, and 12.3% shell, and that the moisture content
377 of albumen is about 87.9% while that of yolk is 48.7% (Romanoff and Romanoff,
378 1949). At 5 mg Se/kg in the diet, the hatching of eggs was "slightly reduced," and Se
379 in egg albumen and yolks averaged about 24 and 5.2 mg/kg dw (2.95 and 2.73
380 mg/kg ww), respectively. At 10 mg Se/kg, hatching decreased to zero, and albumen
381 and yolks contained about 53 and 7.4 mg Se/kg dw (6.40 and 3.92 mg Se/kg ww),
382 respectively. Based on the percentages of albumen and yolk in chicken eggs and the
383 respective percentages of water in albumen and yolk, a Se threshold of about 10
384 mg/kg dw (3 mg/kg ww) in whole eggs was associated with reproductive
385 impairment in the study where chickens were fed 5 mg Se/kg; this threshold is
386 similar to the findings of more rigorous recent studies with mallards.

387 Harmful concentrations of Se in eggs may be of a different magnitude when another
388 chemical form of Se, sodium selenite, is fed to birds. A diet containing 7 mg Se/kg as
389 sodium selenite caused reproductive impairment in chickens but resulted in only
390 about 7.2 and 3.8 mg Se/kg dw (0.87 and 2.02 mg Se/kg ww) in egg albumen and
391 yolk (Ort and Latshaw, 1978).

392 In another study with chickens, a diet containing 8 mg Se/kg as sodium selenite
393 impaired reproduction, and whole eggs contained from about 5.5 to 7.1 mg/kg dw
394 (1.46 to 1.86 mg/kg ww) of Se (Arnold et al., 1973). The chemical form of Se in
395 chicken eggs seems to be different when sodium selenite rather than
396 selenomethionine is fed (Latshaw, 1975; Latshaw and Osman, 1975).

397 In mallards, a dietary concentration of 25 mg Se/kg as sodium selenite impaired
398 reproduction but resulted in a mean of only about 4.3 mg/kg dw (1.3 mg/kg ww) of
399 Se in eggs (Heinz et al., 1987). Therefore, although higher dietary concentrations of
400 sodium selenite than selenomethionine must be fed to mallards to harm
401 reproduction, lower concentrations of Se in eggs are associated with harm.

402 Selenium also may affect egg fertility in some species, but egg fertility is not always
403 reported from field or laboratory studies. Lack of reporting on fertility effects in
404 some studies of Se effects in birds may be due in part to a general practice of simply
405 including infertile eggs as inviable eggs (i.e., "infertility" effects may not be
406 separated from "embryotoxic" effects in the overall measurement of hatchability).
407 Failure to measure infertility as a separate endpoint may be due to the difficulty
408 often associated with distinguishing infertile eggs from those containing embryos
409 that have died very early in development. Nevertheless, decreased fertility is a
410 distinct effect from embryotoxicity, particularly in that it can indicate a mechanism
411 acting on adult, rather than embryonic, physiology. In American kestrels fed
412 selenomethionine at 12 mg Se/kg, egg fertility was significantly reduced (by over
413 14%) compared to kestrels fed 6 mg Se/kg (Santolo et al., 1999). Results obtained in
414 kestrels suggest infertility may be an important factor contributing to the overall
415 reproductive impairment in some species. However, in mallards (Heinz et al., 1987;
416 Heinz and Hoffman, 1996, 1998) and black-crowned night-herons (Smith et al., 1988)
417 fed 10 mg Se/kg as selenomethionine, egg fertility was not reduced compared with
418 controls. Similarly, fertility was not affected in mallards fed diets containing Se at 7
419 mg/kg (Stanley et al., 1996) or 16 mg/kg (Heinz et al., 1989) as selenomethionine,

420 but hatchability of fertile eggs was significantly reduced. Thus, effects on egg
421 fertility in mallards and night-herons are not likely to be as ecologically significant
422 as reduced hatchability.

423 Field Studies

424 Selenium concentrations in the eggs of marine species are variable, but may be
425 higher than in freshwater or terrestrial birds, even in remote areas (Ohlendorf, 1989).
426 For example, eggs of three species (wedge-tailed shearwater [*Puffinus pacificus*], red-
427 footed booby [*Sula sula*], and sooty tern [*Sterna fuscata*]) were sampled at four
428 locations throughout the Hawaiian Archipelago, from Oahu to Midway (Ohlendorf
429 and Harrison, 1986). Mean Se concentrations varied only slightly by location, from
430 about 4.4 to 5.3 mg/kg dw (1.1 to 1.4 mg/kg ww) for shearwaters, 5.0 to 6.1 mg/kg
431 (0.76 to 0.92 mg/kg ww) for boobies, and 4.1 to 5.1 mg/kg (1.1 to 1.4 mg/kg ww) for
432 terns, but all were higher than typical of freshwater species. Henny et al. (1995)
433 predicted egg concentrations (21.3 or 29.2 mg Se/kg dw, based on different
434 regressions) from liver concentrations in white-winged scoters (*Melanitta fusca*)
435 (mean of 54 mg Se/kg dw for combined males and females; concentration not given
436 separately for females) based on established liver-egg relationships for freshwater
437 species (Henny and Herron, 1989; Ohlendorf et al., 1990; Ohlendorf and Hothem,
438 1995). However, they found that Se concentrations in eggs were only about 10% of
439 the predicted concentrations, from 2.7 to 4.7 mg/kg dw.

440 Braune et al. (2002) analyzed eggs of glaucous gulls (*Larus hyperboreus*), black-legged
441 kittiwakes (*Rissa tridactyla*), thick-billed murre (*Uria lomvia*), and black guillemots
442 (*Cepphus grylle*) from the Canadian Arctic. Mean Se concentrations varied somewhat
443 by species and location, with all means between 1.1 and 2.7 mg/kg dw except for
444 kittiwakes (with means of 4.4 mg/kg at two locations), so kittiwakes were the only
445 species with means greater than typical of freshwater and terrestrial birds.

446 Eggs of common eiders (*Somateria mollissima*) collected from five locations in the
447 Baltic Sea near coastal Finland also had median Se concentrations (0.55 mg/kg ww;

448 about 1.65 mg/kg dw) that were similar to background for freshwater and terrestrial
449 birds (Franson et al., 2000). Thus, there seems to be no consistent difference between
450 marine and other birds.

451 Using the results of extensive field studies of black-necked stilts (*Himantopus*
452 *mexicanus*), Skorupa (1998a, 1999) found a threshold of 6 to 7 mg Se/kg in eggs to be
453 associated with impaired egg hatchability. That concentration is about equivalent to
454 the EC₁₀ on a clutch-wise (or hen-wise) basis and the EC₀₃ on an egg-wise basis. Lam
455 et al. (2005) used the same statistical approach as described above for the laboratory
456 study with mallards to estimate the threshold for effects on stilt clutch viability.
457 They derived an EC_{11.8} of 14 mg Se/kg at which clutch viability was significantly
458 impaired (i.e., greater than zero impairment). It should be noted that the
459 background rate of clutch inviability (when Se concentrations in eggs are <6 mg/kg)
460 is estimated at 8.7% (USDI, 1998).

461 Studying birds at Kesterson Reservoir in California, Ohlendorf et al. (1986b) used
462 logistic regression to estimate a 50% chance of embryo death or deformity in
463 American coots (*Fulica americana*) when Se concentrations in eggs were about 18
464 mg/kg dw. The estimated Se concentration causing the same effect in black-necked
465 stilts was 24 mg/kg. The value for eggs of eared grebes (*Podiceps nigricollis*) could
466 not be calculated because even the lowest Se concentration detected in eggs (44
467 mg/kg) was embryotoxic. The logistic approach is best suited to estimate the 50%
468 effect concentration, not the concentrations of Se in eggs at which embryo deaths
469 and deformities begin for each species. These concentrations would obviously be
470 somewhat lower than the 50% effect levels.

471 Skorupa and Ohlendorf (1991) examined the relation between Se concentrations in
472 eggs of various aquatic bird species and reproductive impairment at the population
473 level. Embryo deformities were detected in only 3 of 55 populations of birds that
474 had a mean Se concentration of less than 3 mg/kg in eggs (and these deformities
475 were not characteristic of those induced by Se); this is a concentration of Se judged

476 to represent a background level (Figure 1). However, as discussed earlier, reference
477 area concentrations may not always be the same as concentrations from known
478 uncontaminated areas and, therefore, are not necessarily always synonymous with
479 safe levels. Deformities were detected in 9 of 10 populations of aquatic birds in
480 which the mean Se concentration in eggs exceeded about 48 mg/kg. Their data
481 suggested that a teratogenic threshold at the population level existed between about
482 13 and 24 mg Se/kg, as illustrated in the figure.

483 The nature of Se-related deformities makes them a good measure for characterizing
484 the dose-response relation between Se concentrations in eggs and the incidence of
485 severe reproductive impairment in avian populations because 1) the embryo is
486 either deformed or normal (a presence/absence indicator), and 2) the deformities
487 resulting from Se toxicosis are diagnostic of Se toxicosis. It should be noted,
488 however, that the data plotted in Figure 1 represent a population-level analysis and
489 can not be used to infer probability of teratogenesis in individual eggs of known Se
490 content.

491 Using data on Se in eggs from the Tulare Basin (southern San Joaquin Valley),
492 combined with data from several other western sites where elevated Se was found,
493 Skorupa (1998 a, b; also in USDI, 1998) documented a detailed exposure-response
494 relationship. Statistically distinct teratogenesis response functions were delineated
495 for ducks, stilts, and American avocets (*Recurvirostra americana*) using the Tulare
496 Basin data. The Tulare curves were used to estimate expected frequencies of
497 teratogenesis for ducks, stilts, and avocets using other sites, and the predicted levels
498 were tested against the observed frequencies from the sites. The predicted and
499 observed frequencies of teratogenesis were not significantly different, so the data
500 were combined to generate final response curves. Using these data, Skorupa (1998b)
501 developed species-specific response curves for stilts and avocets and a composite
502 duck curve (using combined data from gadwalls [*Anas strepera*], mallards, pintails
503 [*A. acuta*], and redheads [*Aythya americana*]).

504 Based on the response coefficients and their standard errors, the teratogenesis
505 function for ducks, stilts, and avocets were significantly different (Skorupa, 1998b).
506 Within this data set, these responses represent "sensitive" (duck), "average" (stilt),
507 and "tolerant" (avocet) species. The probability of overt teratogenesis in stilts
508 increased markedly when Se concentrations in eggs were greater than 40 mg/kg,
509 with an EC₁₀ for teratogenic effects of 37 mg/kg. In contrast, the thresholds for
510 teratogenesis (expressed as an EC₁₀) were 23 mg Se/kg in mallards and 74 mg Se/kg
511 in avocets. Sensitivity of these species to effects of Se on egg hatchability followed a
512 similar pattern, with mallards being more sensitive than stilts, which are more
513 sensitive than avocets (USDI, 1998).

514 **Liver**

515 Background Se concentrations in livers of freshwater and terrestrial birds are <10
516 mg/kg dw (Table 1), while livers of marine birds from uncontaminated areas tend to
517 have considerably higher Se concentrations (often 20 mg/kg or more; Dietz et al.,
518 1996; Trust et al., 2000; Grand et al., 2002; Mallory et al., 2004; Elliott, 2005). Typical
519 moisture content is about 70% (Ohlendorf et al., 1990; Stanley et al., 1996).

520 Laboratory Studies

521 In a manner similar to that for eggs, Se concentrations in the liver respond quickly
522 when birds are placed on or taken off a Se-contaminated diet (Heinz et al., 1990).
523 When mallards were fed a diet containing 10 mg Se/kg, Se concentrations in liver
524 were predicted to reach 95% of equilibrium in 7.8 days; the rate of loss from liver
525 also was rapid, with half-time of 18.7 days. Thus, Se concentrations measured in the
526 livers of birds sampled outside the breeding season are not good predictors of
527 potential reproductive effects. In laboratory studies of reproductive effects, livers of
528 male mallards had higher concentrations of Se than those of females, probably
529 because females excreted part of the Se they had accumulated through egg-laying
530 (e.g., Heinz et al., 1987, 1989; Heinz and Hoffman, 1998). Nevertheless, analysis of

531 livers of either male or female field-collected birds can provide a useful indication of
532 the relative level of exposure experienced by the population.

533 Laboratory studies have been conducted with mallards to determine the kinds of
534 lesions and other measurements that can be used for diagnosis of Se toxicosis in
535 birds (Albers et al., 1996; Green and Albers, 1997; O'Toole and Raisbeck, 1997, 1998).
536 Dietary concentrations of added Se ranged from 10 to 80 mg/kg in these studies.
537 Various hepatic lesions were associated with dietary exposures greater than 10 mg
538 Se/kg, and Se concentrations in livers increased in response to the dietary levels. In
539 general, ducks that received diets containing more than 20 mg Se/kg developed a
540 number of lesions of the liver, and those receiving 40 mg/kg or more Se in their
541 diets lost weight and had abnormal changes in the integument (described below) in
542 addition to the liver. Lesions of the integument and liver, and weight loss, when
543 corroborated by elevated Se concentrations in tissues (especially the liver), can be
544 diagnostic of Se toxicosis in birds. It should be noted, however, that some birds died
545 without exhibiting any significant morphological lesions even though they were
546 emaciated. Although a clear threshold Se concentration in livers (or other tissues) for
547 diagnosis of Se toxicity could not be defined, concentrations greater than 10 mg/kg
548 were considered suspicious of Se toxicosis, particularly when accompanied by
549 emaciation, poor quality (and sloughing) of nails, bilaterally symmetrical alopecia of
550 the head and neck, toxic hepatic lesions, and necrosis of maxillary nails.

551 In laboratory studies with birds fed diets containing selenomethionine, when Se
552 concentrations in the diet and in livers of mallards, night-herons, and eastern
553 screech-owls were expressed on a dry-weight basis, liver concentrations ranged
554 from roughly equal to the dietary concentrations to about three times the dietary
555 levels (Heinz et al., 1987, 1989; Smith et al., 1988; Stanley et al., 1994, 1996; Wiemeyer
556 and Hoffman, 1996). At Kesterson Reservoir, Se concentrations in livers of European
557 starling (*Sturnus vulgaris*) nestlings (7.5 mg/kg) were only slightly higher than those
558 in the invertebrates being fed to the chicks (6.2 mg/kg) by adults (Santolo, 2007).

559 In a laboratory study, surviving mallard ducklings fed 40 mg Se/kg as
560 selenomethionine had a mean Se concentration of about 224 mg/kg dw (68 mg/kg
561 ww) in the liver, whereas ducklings that died had a mean of about 198 mg/kg dw
562 (60 mg/kg ww) (Heinz et al., 1988). In another laboratory study, this time with adult
563 male mallards fed 100 mg Se/kg as selenomethionine, the livers of survivors
564 contained a mean of about 142 mg Se/kg dw (43 mg Se/kg ww), and the livers of
565 birds that died contained a mean of about 125 mg Se/kg dw (38 mg Se/kg ww)
566 (Heinz, 1993a).

567 When adult male mallards were fed 32 mg Se/kg as selenomethionine, they
568 accumulated an average of about 96 mg Se/kg dw (29 mg Se/kg ww) in their livers
569 (Hoffman et al., 1991). One of 10 birds fed 32 mg Se/kg died, and others had
570 hyperplasia of the bile duct and hemosiderin pigmentation of the liver and spleen.
571 Various other sublethal effects, such as elevated plasma alkaline phosphatase
572 activity and a change in the ratio of hepatic oxidized glutathione to reduced
573 glutathione, were observed in ducks with lower hepatic concentrations. At a dietary
574 concentration of 8 mg Se/kg, which caused several of the physiological effects
575 mentioned above, the mean concentration of Se in the liver was about 41 mg/kg dw
576 (12.5 mg/kg ww).

577 Based on these laboratory studies, in which Se was present as selenomethionine in
578 the diet and was the only element fed at toxic concentrations, mortality of young
579 and adult mallards could occur when hepatic concentrations of Se reach roughly 66
580 mg/kg dw (20 or more mg/kg ww), and important sublethal effects are likely when
581 the concentrations exceed about 33 mg/kg dw (10 mg/kg ww).

582 Using Se concentrations in adult female livers to predict when reproductive
583 impairment occurs in birds is not nearly as good as using Se concentrations in eggs,
584 because it is the Se in the egg that actually harms the embryo (Skorupa and
585 Ohlendorf, 1991). Extrapolating from liver to egg will introduce additional
586 uncertainty above that already existing for the egg. However, in a controlled

587 laboratory study, the correlation between Se concentrations in eggs and in the livers
588 of laying females was demonstrated by feeding mallards selenomethionine (Egg
589 $\text{Se}_{\text{mg/kg ww}} = -1.10 + 2.6 (\text{Liver Se}_{\text{mg/kg ww}})$; $R^2 = 0.83$; $P < 0.01$; Heinz et al., 1989).
590 Therefore, when Se concentrations in eggs are not available, the concentrations in
591 the livers of females during the breeding season can be used to estimate whether
592 reproduction might be impaired. When Se concentrations are known for both the
593 eggs and livers of breeding females, judgments on the hazards of Se to reproduction
594 should be based on Se in the egg.

595 In laboratory studies of reproduction, the livers of male mallards contained more Se
596 than did the livers of females fed the same diets (Heinz et al., 1987; Heinz et al.,
597 1989). Because females may use the egg as a route of Se excretion unavailable to
598 males, one would expect that, in the field, the lowest reproductive effect threshold of
599 Se would be in the livers of laying females and that the livers of males would be less
600 useful in predicting effects on reproduction, even if the males were collected during
601 the breeding season and from the area where reproduction is of concern. The
602 advantage of sampling laying females, however, may be more academic than
603 practical. In nature, it is easier and more likely that a female would be collected
604 before or after egg laying, at which time the concentration of Se in her liver should
605 be the same as in the liver of a male. If one collects breeding males in the wild or has
606 reason to believe that the collected females were not collected during egg laying, a
607 10-mg/kg dw (3-mg/kg ww) threshold concentration of Se in the liver would be on
608 the low side (and would represent the upper end of background conditions); a value
609 of about 13 to 20 mg Se/kg dw (4 to 6 mg Se/kg ww) might be more appropriate for
610 freshwater birds. However, some marine species typically have higher hepatic Se
611 concentrations even in remote areas (as noted previously), so these values would not
612 be appropriate for those species.

613 Female mallards that were fed 10 mg Se/kg as selenomethionine had reduced
614 reproductive success and a mean of about 16 mg Se/kg dw (4.7 mg Se/kg ww) in

615 their livers (Heinz et al., 1987). Because no dietary concentrations below 10 mg/kg
616 were used, a no-effect level of Se in the liver was not determined in this study.

617 A dietary concentration of 8 mg Se/kg as selenomethionine significantly reduced
618 reproductive success of mallards, and livers of the treated females contained a mean
619 of about 12 mg Se/kg dw (3.5 mg Se/kg ww) (Heinz et al., 1989). In the same study,
620 reproductive success was not significantly different between females fed 4 mg Se/kg
621 and controls, and livers contained a mean of about 7.9 mg Se/kg dw (2.4 mg Se/kg
622 ww). Based on a regression equation of Se concentrations in female livers versus
623 their eggs (Heinz et al., 1989), the threshold Se concentration of 10 mg/kg dw (3
624 mg/kg ww) in eggs corresponds to a Se value of about 5.3 mg/kg dw (1.6 mg/kg
625 ww) in the liver. However, we do not know whether the data for this regression
626 were linear in the lower end of the Se range. If the data were curvilinear, a value of
627 10 mg Se/kg dw (3 mg Se/kg ww) in eggs may correspond to a value of roughly 10
628 mg Se/kg dw (3 mg Se/kg ww) for the liver.

629 In these laboratory studies with mallards, between 16 and 31 eggs were laid before
630 each female was sacrificed. Depletion of Se through egg laying, therefore, may have
631 been greater in the laboratory than in nature where birds lay fewer eggs. If depletion
632 of Se is greater by females in a laboratory study, the Se concentrations in the liver
633 associated with reproductive impairment could be on the low side.

634 Separate studies were conducted to evaluate the interactive effects of Se with As
635 (Stanley et al., 1994), B (Stanley et al., 1996), and Hg (Heinz and Hoffman, 1998). The
636 results of the interactions are described in more detail in a later section
637 (Interactions); here we discuss only the effects of the Se treatment by itself. When Se
638 was fed alone at dietary concentrations of 3.5 or 7.0 mg/kg in the B study, the mean
639 Se concentration in livers of females was about 11 mg/kg dw (3.5 mg/kg diet) or 17
640 mg/kg (7 mg/kg diet) (3.2 and 5.1 mg/kg ww in liver). Hatching success was
641 reduced in the 7-mg Se/kg treatment group when compared to controls and the 3.5-
642 mg Se/kg treatment group. No embryonic deformities were found in that study;

643 although Se reduced duckling weight, it did not affect duckling survival. When
644 ducks were fed Se at 10 mg/kg in both the As and Hg studies, Se accumulated
645 significantly in eggs and livers, reduced hatching success and duckling survival (or
646 production per pair), and was teratogenic. In the As study, the mean Se
647 concentration in livers of ducks receiving the 10-mg/kg diet was 31 mg/kg in
648 females and 34 mg/kg in males. In the Hg study, the mean Se concentration in livers
649 of hens receiving the 10-mg/kg diet was about 20 mg/kg dw (6.0 mg/kg ww), and
650 in males it was about 32 mg/kg dw (9.6 mg/kg ww).

651 Franson et al. (2007) fed common eiders a diet containing 20 mg Se/kg as seleno-L-
652 methionine or a diet that was started at 20 mg Se/kg and increased over time to 60
653 mg Se/kg. Among the ducks fed the 20-mg Se/kg diet, 57% exhibited lipidosis and
654 hypertrophy of Kupffer cells in the liver. Among the ducks fed the 60-mg Se/kg
655 diet, 83% exhibited cellular lipidosis and 100% had hypertrophy of Kupffer cells.
656 One duck in the 60-mg Se/kg group died after 30 days and another was euthanized
657 on day 32 after developing a staggering gait and a 35% weight loss. Selenium
658 concentrations in livers averaged 351 mg/kg dw in the 20-mg/kg dietary group and
659 735 mg/kg dw in the 60-mg/kg dietary group. The authors of that study stated that
660 the effects of Se generally were comparable to those seen in mallards fed similar
661 dietary concentrations of selenomethionine; however, the eiders accumulated more
662 Se in their livers than did the mallards. For example, in one study (O'Toole and
663 Raisbeck, 1997) mallards fed 60 mg Se/kg accumulated about 200 mg Se/kg dw
664 (60.6 mg Se/kg ww) in liver versus the 735 mg Se/kg dw for the eiders fed 60 mg
665 Se/kg in the Franson et al. (2007) study, leading the authors of the eider study to
666 conclude that eiders, and probably other sea ducks, apparently have a higher
667 adverse effects threshold of Se in tissues than do freshwater species.

668 Field Studies

669 Selenium concentrations in the liver have been used to estimate both exposure and
670 effects on birds. For example, livers of adult birds (coots, stilts, and ducks) collected

671 from Kesterson Reservoir and reference areas showed time-period differences
672 related to collection site and duration of exposure (Ohlendorf et al., 1990). In
673 addition, Se concentrations in pre-fledging juvenile birds of some species were
674 generally similar to those in livers of late-season adults. Geometric means for Se in
675 adult stilts in 1983 were as follows: Kesterson Reservoir - 41.8 mg/kg early, 94.4
676 mg/kg late nesting season; Volta Wildlife Area - 10.7 mg/kg early, 5.41 mg/kg late
677 nesting season. Selenium concentrations in juveniles were 94.6 mg/kg at Kesterson
678 and 4.10 mg/kg at the Volta Wildlife Area.

679 Although accumulation in the liver is dose-dependent (Hoffman et al., 1991), the
680 hepatic concentration is only an imprecise estimator of the pathological condition of
681 a bird. The cutoff is not clear between Se concentrations in the livers of birds killed
682 by Se poisoning and others exposed to high concentrations but collected alive. The
683 livers of birds found dead at the Kesterson Reservoir contained 26 to 86 mg Se/kg,
684 whereas the livers of birds shot there contained 38 to 85 mg Se/kg (Ohlendorf et al.,
685 1988).

686 Selenium toxicosis effects in several species of aquatic birds found at Kesterson
687 Reservoir in 1984-1986 were described previously (Ohlendorf 1989, 1996; Ohlendorf
688 and Hothem, 1995; Ohlendorf et al., 1988, 1990). Those birds exhibited many of the
689 same signs of selenosis as those later found in mallards (as described below),
690 including hepatic lesions, alopecia, necrosis of the beak, and weight loss.

691 Livers of diving ducks (such as scoters [*Melanitta* spp.] and scaups [*Aythya* spp.])
692 from estuarine habitats have been found to contain higher concentrations of Se than
693 other aquatic birds in the same habitats (Ohlendorf et al., 1986c, 1989, 1991; Henny et
694 al., 1991). One possible reason for the higher concentrations of Se in these diving
695 ducks is that they forage on benthic organisms, which bioaccumulate Se to a higher
696 degree than foods of some other aquatic birds. However, many species of marine
697 birds, including some that feed on planktonic crustaceans or other near-surface
698 organisms, also tend to have higher hepatic Se concentrations than typical of

699 freshwater birds (Elliott et al. 1992; Dietz et al., 1996; Campbell et al., 2005; Elliott,
700 2005). Those include species such as Leach's storm-petrel (*Oceanodroma leucorhoa*),
701 northern fulmar (*Fulmarus glacialis*), black-footed albatross (*Diomedea nigripes*), and
702 black-legged kittiwake that have mean Se concentrations up to 75 mg/kg.

703 Based on field data, a very high risk of embryonic deformity exists when the mean
704 Se concentration in the livers of a population of birds using non-marine habitats
705 (both sexes included and females not necessarily laying) exceeded about 30 mg/kg
706 dw (U.S. Fish and Wildlife Service, 1990). Populations with means below about 10
707 mg Se/kg dw generally did not have many deformed embryos. Some species of
708 marine birds can accumulate high concentrations of Se in their livers without
709 correspondingly high concentrations in their eggs (e.g., Henny et al., 1995; Braune et
710 al. 2002; Campbell et al., 2005; DeVink et al. 2008b)

711 **Kidney**

712 Background Se concentrations in bird kidneys have not been clearly defined, and
713 there is no consistent trend regarding liver/kidney ratios. Selenium concentrations
714 in kidneys of birds from Se-normal areas were somewhat higher than those in the
715 liver (liver/kidney ratios of less than 1), but concentrations in the two tissues were
716 similar in birds from the Se-contaminated Kesterson Reservoir (Ohlendorf et al.,
717 1988, 1990) and in the Imperial Valley of California (Koranda et al., 1979). Selenium
718 concentrations in liver and kidneys of American coots from Kesterson Reservoir and
719 the reference site (Volta Wildlife Area) were significantly correlated ($r = 0.98$). The
720 average moisture content of kidneys was 76-78%, so a conversion factor of 4.3 can be
721 used to estimate from wet-weight to dry-weight concentrations.

722 When chickens were fed 0.1 mg Se/kg as selenomethionine for 18 weeks, Se
723 concentrations in kidneys (about 3.3 mg/kg dw; 0.77 mg/kg ww) were higher than
724 those in the liver (about 2.0 mg/kg dw; 0.60 mg/kg ww), but when the diet
725 contained 6 mg Se/kg the kidney and liver Se concentrations were essentially equal

726 (both about 22 mg/kg dw; 5.2 and 6.6 mg/kg ww, but with different moisture
727 contents assumed for kidney and liver) (Moksnes, 1983).

728 In a study to determine body distribution of trace elements in black-tailed gulls
729 (*Larus crassirostris*) nesting on Rishiri Island in Hokkaido Prefecture, Japan, Se
730 concentrations in kidneys of both adults (6.9 mg/kg) and juveniles (6.5 mg/kg) were
731 significantly ($P < 0.001$) higher than in livers (adults, 4.5 mg/kg; juveniles, 5.3
732 mg/kg) (Agusa et al., 2005).

733 In a laboratory study with mallards (Albers et al., 1996), Se concentrations in livers
734 of surviving ducks were consistently higher than those in kidneys when the ducks
735 were fed diets supplemented with Se at 0 (control), 10, 20, or 40 mg/kg. However,
736 concentrations in the two tissues were more similar among the birds that died
737 during the exposure period. When expressed on a dry-weight basis, Se
738 concentrations in livers were about two or three times the dietary concentration,
739 whereas those in kidneys averaged less than twice the dietary concentration.

740 Although concentrations of Se in kidneys representative of those diagnostic of harm
741 to adult health or reproductive success are poorly understood, if one had no other
742 information on Se values in tissues other than in kidneys, one could assume a
743 roughly one-to-one correspondence between the concentration of Se in kidney and
744 liver. In this way one could make a preliminary assessment of possible harm to
745 birds, but this assessment would be weak compared to those based on
746 concentrations in eggs or livers.

747 **Muscle**

748 Background Se concentrations in muscle tissues of birds are 1-3 mg/kg (Table 1).
749 Average moisture content of mallard muscle in a laboratory study was 74% (Heinz
750 et al., 1987).

751 As in eggs and liver, Se concentrations in muscle increase and decrease in response
752 to changes in dietary exposure, but the changes occur more slowly (Heinz et al.,

1990) and diagnostic concentrations for effects are not readily available. Heinz et al. (1990) fed female mallards 10 mg Se/kg as selenomethionine for 6 weeks, followed by 6 weeks off treatment, and measured Se in the liver and breast muscle. By 6 weeks, Se in breast muscle averaged about 24 mg/kg dw (6.3 mg/kg ww). Selenium in the liver had nearly peaked after about 1 week, whereas muscle was projected to reach a peak of about 30 mg Se/kg dw (8 mg Se/kg ww) after 81 days. Likewise, Se was eliminated faster from the liver than from breast muscle, indicating that the two tissues may contain similar concentrations of Se, but only after both reach equilibrium. This difference in accumulation and loss rates between tissues helps explain the variability observed in the muscle-liver relationships at Kesterson Reservoir and the reference site described below (Ohlendorf et al., 1990).

Selenium concentrations in breast muscle from juvenile ducks (*Anas* spp.) at Kesterson Reservoir and a reference site (Volta Wildlife Area) were measured because of concern about human consumption of ducks harvested in the vicinity of Kesterson (Ohlendorf et al., 1990). Mean Se concentrations were higher at Kesterson than the reference site, and were only slightly lower than those in livers of these birds. However, the relationship between muscle and liver ($R^2 = 0.69$) of the ducks was considerably more variable than that between kidneys and livers of American coots from the two sites ($R^2 = 0.97$). The predictive equation was:

$$\text{Log Se in muscle} = 0.22 + 0.65 \text{ log Se in liver.}$$

When mallards were fed 10 mg Se/kg as selenomethionine in a laboratory study, females had similar concentrations of Se in the liver (about 16 mg/kg dw; 4.7 mg/kg ww) and breast muscle (about 19 mg/kg dw; 4.9 mg/kg ww), whereas males had much higher concentration in the liver (about 28 mg/kg dw; 8.6 mg/kg ww) than in breast muscle (about 12 mg/kg dw; 3.1 mg/kg ww) (Heinz et al., 1987). Because the females were laying eggs, they may have been using stores of Se from the liver to incorporate into eggs.

780 Fairbrother and Fowles (1990) reported more Se in breast muscle (about 22 mg/kg)
781 than in the liver (about 16 mg/kg) of male mallards given drinking water containing
782 2.2 mg Se/L (as selenomethionine) for 12 weeks. When chickens were fed 0.1 mg
783 Se/kg as selenomethionine for 18 weeks, Se concentrations in breast muscle (about
784 1.1 mg/kg dw; 0.29 mg/kg ww) were about half of those in the liver (about 1.9
785 mg/kg dw; 0.60 mg/kg ww), but when fed 6 mg Se/kg in the diet nearly equal Se
786 concentrations were reported in the breast muscle and liver (20 and 22 mg/kg dw;
787 5.4 and 6.6 mg/kg ww) (Moksnes, 1983).

788 As was the case with liver, much more Se was accumulated in muscle when ducks
789 received an organic form of Se (selenomethionine) at 10 mg/kg than when fed a diet
790 supplemented with an equivalent concentration of inorganic Se (selenite, which is
791 used routinely, but at much lower concentrations, in poultry diets) (Heinz et al.,
792 1987). Also, females that received the organic Se during the reproductive study
793 accumulated significantly more Se in breast muscle than the males receiving the
794 same treatment.

795 **Blood**

796 Background Se concentrations in whole blood of non-marine birds are 0.1-0.4 mg/L
797 on a wet-weight basis (Table 1). However, marine birds inhabiting unpolluted areas
798 often have higher Se concentrations in their blood (e.g., Franson et al., 2000;
799 Wayland et al., 2001, 2008; Grand et al., 2002), and similar findings were observed at
800 Great Salt Lake, UT (Conover and Vest, 2009).

801 Under uniform sampling conditions, the moisture content of blood is fairly uniform,
802 but under field conditions the moisture content can vary substantially. For example,
803 when mallard blood was sampled over a period of about 3 months by
804 exsanguination in a laboratory study, the dry-weight content of blood averaged
805 $21.70 \pm 0.21\%$ (mean \pm SE) (Scanlon, 1982). In a laboratory study with kestrels
806 (Yamamoto et al., 1998; Santolo et al., 1999; G.M. Santolo, pers. com.), the dry-weight
807 content of blood averaged $21.40 \pm 0.11\%$ (mean \pm SE) with a range from 14 to 25%.

808 However, when kestrels and other raptors were sampled in the field (Santolo and
809 Yamamoto, 1999; G.M. Santolo, pers. com.), the dry-weight content of blood
810 averaged $19.30 \pm 0.14\%$ (mean \pm SE) with a range from 9 to 32%. In both the
811 laboratory and field studies of kestrels (and other raptors), blood samples were
812 taken in a consistent manner from the birds by the same investigators. However,
813 there was much greater variability in moisture content of birds collected in the field
814 (Variance = 8.3) and than in the lab (Variance = 2.2).

815 In experimental studies, Se concentrations in blood of mallards (Heinz et al., 1990;
816 Heinz and Fitzgerald, 1993a; O'Toole and Raisbeck, 1997) and American kestrels
817 (Yamamoto et al., 1998; Santolo et al., 1999) reflected dietary exposure levels.
818 Mallards receiving Se (as selenomethionine) at dietary concentrations of 10, 25, or 60
819 mg/kg had blood-Se concentrations of about 50, 125, or 300 mg/L dw (4.5, 8.9, or 16
820 mg/L ww) (O'Toole and Raisbeck, 1997). The concentration of Se in blood increased
821 in a time- and dose-dependent manner and reached a plateau after 40 days.

822 When female mallards were fed increasingly high dietary concentrations of Se as
823 selenomethionine (from 10 mg/kg to 160 mg/kg over a period of 31 days), birds
824 began to die at the end of the 31-day exposure (Heinz et al., 1990). Survivors
825 contained means of about 60 mg Se/kg dw (12 mg Se/kg ww) in the blood on day
826 31, when their diet was switched to an untreated diet. Half-time for loss of Se from
827 blood was 9.8 days, which was much faster than for muscle (23.9 days). In another
828 study (Heinz and Fitzgerald, 1993a), adult male mallards were fed 10, 20, 40, or 80
829 mg Se/kg as selenomethionine. Mortality began in the 40- and 80-mg Se/kg
830 treatment groups during the third week on treatment, when samples of blood from
831 surviving ducks in the same pens contained means of about 25 or 70 mg Se/kg dw
832 (5 or 14 mg Se/kg ww). Blood Se concentrations of the ducks fed lower-Se diets
833 plateaued after 8 weeks at about 42 mg/kg dw (8.4 mg/kg ww) for the 10-mg/kg
834 treatment group and 70 mg/kg dw (14 mg/kg ww) for the 20-mg/kg dietary
835 concentration. However, samples of blood were not taken from any of the birds that

836 died. Therefore, comparisons of Se concentrations between the dead and the
837 survivors were not possible.

838 In American kestrels (Yamamoto et al., 1998), maximal blood concentrations, when
839 expressed on a dry-weight basis, were about the same as those in the
840 selenomethionine-supplemented diet. The Se concentration in blood after 77 days on
841 treatment was 5.0 mg/kg for kestrels receiving the 5 mg/kg diet and 8.9 mg/kg for
842 those receiving the 9 mg/kg dietary concentration. Selenium concentrations in blood
843 returned to near the control concentrations in 28 days after the experimental diets
844 were removed. Selenium concentrations in excreta of the kestrels were higher than
845 those in blood during the treatment period, indicating that they excrete a substantial
846 amount of the ingested Se.

847 To assess the possible effects of Se on reproduction and fitness (measured as body
848 mass) of lesser scaup, captive scaup were fed a control diet or one supplemented
849 with Se at 7.5 or 15 mg/kg for 30 days to simulate late spring migration (DeVink et
850 al., 2008a). The treated feed was removed after 30 days, before the birds began
851 laying. There was no effect of Se on body mass, breeding probability, or clutch
852 initiation dates. Blood Se concentrations differed between the treatment groups in
853 proportion to dose, with mean Se concentrations in blood after 30 days on treatment
854 (16.3 and 30.8 mg/kg) about twice the concentration in the diet. The half-lives for Se
855 concentrations in blood were 22 days for the 7.5-mg/kg treatment group and 16
856 days for the 15-mg/kg treatment group.

857 When Franson et al. (2007) fed common eiders a diet containing 20 mg Se/kg as
858 seleno-L-methionine or a diet that was started at 20 mg Se/kg and increased over
859 time to 60 mg Se/kg (as described in Liver section), the eiders accumulated high
860 concentrations of Se in their blood. Within 35 days on the high-Se diet the eiders lost
861 about 30% of their body mass and mean blood Se concentration was about 88 mg/kg
862 (17.5 mg/kg ww). Body mass of the eiders on the 20-mg Se/kg diet was similar to
863 that of controls, although mean blood Se in the 20-mg/kg group was about 70

864 mg/kg (14 mg/kg ww), which was higher than that of controls (about 2 mg/kg;
865 <0.4 mg/kg ww).

866 Differences in the relationship between blood and liver Se concentrations may be
867 attributed to more rapid initial elimination from liver than blood (Heinz et al., 1990;
868 Wayland et al., 2001) and to binding of Se to inorganic mercury (IoHg) forming an
869 inert Hg-Se protein with a long half-life (Scheuhammer et al., 1998).

870 Selenium concentrations in wild-trapped birds can be measured in blood as a non-
871 lethal approach for assessing exposure and, when combined with laboratory
872 findings, can be interpreted as to whether exposures are potentially harmful. For
873 example, Se concentrations were measured in terrestrial birds of several species
874 from Kesterson Reservoir, the area surrounding that site, and several reference areas
875 in California from 1994 to 1998 (Santolo and Yamamoto, 1999). Except for
876 loggerhead shrikes (*Lanius ludovicianus*), blood-Se was higher in birds from within
877 Kesterson than in birds from other areas. For shrikes, the mean Se concentrations for
878 birds from Kesterson (13 mg/kg dw) were not significantly different than those
879 from nearby surrounding areas (8.5 mg/kg), although the maximum Se
880 concentration at Kesterson (38 mg/kg) was more than twice the maximum for the
881 surrounding area (16 mg/kg). Among species at Kesterson Reservoir, blood-Se
882 concentration was higher in loggerhead shrikes and northern harriers (*Circus*
883 *cyaneus*) than in the other species (hawks and owls) sampled. This difference among
884 species is likely due to the differing sizes of foraging ranges of the various species
885 (nesting harriers and young were sampled). Adult starlings collected from nest
886 boxes within Kesterson had a mean Se concentration of 16 mg/kg in blood, and
887 concentrations in eggs were significantly correlated with those in blood (Santolo,
888 2007).

889 Based on the information available, we conclude that Se concentrations in blood can
890 indicate recent dietary exposures of birds, but relationships vary among species, and

891 concentrations in blood can not be clearly related to effects on reproduction or
892 individual health and fitness.

893 **Integument/Feathers**

894 Background concentrations of Se in feathers are 1-4 mg/kg, and are typically less
895 than 2 mg/kg (Table 1), with moisture content of about 10%. As is the case for liver
896 and other tissues, Se concentrations may be higher in the feathers of birds from areas
897 with elevated levels of Hg, because of the interactions between these two elements.
898 Analyses of feathers may provide useful information concerning exposures of birds
899 to Se if they are considered carefully. It is important to recognize that the Se may
900 have been deposited into the feathers at the time they were formed (which may have
901 been months earlier and thousands of miles away from the sampling time and
902 location), or the Se may be the result of external contamination (Goede and de Bruin,
903 1984, 1985, 1986; Goede et al., 1989; Burger, 1993). Concentrations also may have
904 been reduced through leaching. Different kinds of feathers from the same bird may
905 contain different concentrations, depending partly on when and where the feathers
906 were grown during the molt cycle.

907 Overall, feathers are not very useful for diagnosing potential harm in birds,
908 especially because Se concentrations in them are not good indicators of current or
909 recent exposure (unless, perhaps, while the feathers are growing) (Burger, 1993;
910 Ohlendorf, 1993; USDI, 1998; Eisler, 2000). However, a Se concentration of 5 mg/kg
911 was identified as a threshold warranting further study (USDI, 1998).

912 Feather loss (bilateral alopecia) is one of the signs of chronic selenosis in birds that
913 may be observed in the field when dietary concentrations are high (Ohlendorf et al.,
914 1988; Ohlendorf, 1996). As mentioned above, laboratory studies have been
915 conducted with mallards to determine the kinds of lesions and other measurements
916 that can be used for diagnosis of Se toxicosis in birds (Albers et al., 1996; Green and
917 Albers, 1997; O'Toole and Raisbeck, 1997, 1998). In general, ducks that received diets
918 containing more than 20 mg Se/kg developed a number of lesions of the

919 integument. Those receiving 40 mg/kg or more Se in their diets lost weight and had
920 abnormal changes in the integument that involved structures containing hard
921 keratin, such as feathers (alopecia/ deptylation [i.e., feather loss]), beaks (necrosis),
922 and nails (onychoptosis [sloughed or broken]). When corroborated by elevated Se
923 concentrations in tissues (especially the liver), the observed integumentary and
924 hepatic lesions, as well as weight loss, can serve for diagnosis of Se toxicosis in birds.
925 It should be noted, however, that some birds died without exhibiting any significant
926 morphological lesions even though they were emaciated.

927 In conclusion, Se concentrations in feathers can indicate exposure of birds at the time
928 the feathers grew, but concentrations that may be diagnostic of problems have not
929 been developed.

930 **Biomarkers**

931 **Biochemical**

932 A number of studies have described physiological changes that are associated with
933 Se exposure in field-collected or laboratory-exposed birds (Ohlendorf et al., 1988;
934 Hoffman and Heinz, 1998; Hoffman et al., 1989, 1991, 1998). These generally
935 involved changes in measurements associated with liver pathology and glutathione
936 metabolism (e.g., glycogen, protein, total sulfhydryl and protein-bound sulfhydryl
937 concentrations; and glutathione peroxidase activity). In lesser scaup, results of a
938 field study suggested that corticosterone release may be influenced by complex
939 contaminant interactions in relation to body condition and body size (Pollock and
940 Machin, 2009). When cadmium concentrations were high and birds were in good
941 body condition, there was a negative relationship between liver Se and
942 corticosterone, but not in birds with poor body condition. The overall mean Se
943 concentration in livers was 4.3 mg/kg, with no apparent difference between the two
944 groups.

945 Wayland et al. (2002) found an inverse association between stress response
946 (measured as corticosterone concentrations following capture) and Se in common

947 eiders nesting in the Canadian Arctic in 1999. Following capture and blood
948 sampling, the birds were placed in a flight pen on-site for 8 days to examine immune
949 function. Cell-mediated immunity was positively related to hepatic Se (geometric
950 means were 14.1 mg/kg in females, 32.1 mg/kg in males). The
951 heterophil:lymphocyte ratio was inversely related to hepatic Se. In 1998, hepatic Se
952 (geometric mean of 17.2 mg/kg in females) was positively related to body mass,
953 abdominal fat mass, kidney mass, and liver mass.

954 Hoffman (2002) and Spallholz and Hoffman (2002) provide discussions of the
955 mechanisms and role of Se toxicity and oxidative stress in aquatic birds. As dietary
956 and tissue concentrations of Se increase, increases in plasma and hepatic glutathione
957 peroxidase activities occur, followed by dose-dependent increases in the ratio of
958 hepatic oxidized to reduced glutathione, and ultimately hepatic lipid peroxidation.
959 At a given tissue (or egg) Se concentration, one or more of these oxidative effects
960 were associated with teratogenesis (at about 15 mg Se/kg dw [4.6 mg Se/kg ww] in
961 eggs), reduced growth of ducklings (at about 50 mg Se/kg dw [15 mg Se/kg ww] in
962 liver), diminished immune system (at about 16 mg Se/kg dw [5 mg Se/kg ww] in
963 liver) and histopathological lesions (at about 96 mg Se/kg dw [29 mg Se/kg ww] in
964 liver) in adults. These effects have been documented in field and laboratory studies,
965 as reviewed by Hoffman (2002).

966 **Morphological**

967 The characteristic reproductive effects of Se observed in both field and laboratory
968 studies include reduced hatchability of eggs (due to embryo mortality) and a high
969 incidence of embryo deformities (teratogenic effects) (Ohlendorf, 1996, 2003).
970 Selenium-induced abnormalities are often multiple and include defects of the eyes
971 (microphthalmia = abnormally small eyes; possible anophthalmia = missing eyes),
972 feet or legs (amelia = absence of legs; ectrodactylia = absence of toes), beak
973 (incomplete development of the lower beak, spatulate narrowing of the upper beak),
974 brain (hydrocephaly = a swelling of the skull due to fluid accumulation in the brain;

975 exencephaly = an opening in the skull that exposes the brain), and abdomen
976 (gastroschisis = an opening of the gut wall, exposing the intestines and other
977 internal organs). Most of these abnormalities are illustrated through photographs
978 that have been published elsewhere (e.g., Ohlendorf et al., 1986a, 1988; Ohlendorf,
979 1989, 1996; Ohlendorf and Hothem, 1995; O'Toole and Raisbeck, 1998).

980 Morphological changes in adult birds as a result of chronically consuming diets with
981 excessive Se have been documented in field and laboratory studies, as described in
982 earlier sections and other reviews (e.g., O'Toole and Raisbeck, 1998; Eisler 2000;
983 Ohlendorf, 1989, 1996, 2003). They include poor body condition (i.e., weight loss and
984 loss of body lipids), feather loss, and histopathological changes in tissues. Tissue
985 concentrations that cause these changes are not clear-cut, but effects are sometimes
986 observed when hepatic Se is >10 mg/kg. American kestrels fed a diet containing Se
987 at a concentration of 12 mg/kg lost lean body mass, suggesting that they were
988 burning muscle mass as a result of this exposure (not seen in the lower treatment
989 group fed 6 mg/kg); this may be the cause of wasting seen in other species
990 (Yamamoto and Santolo, 2000).

991 **Interactions**

992 The most studied interactions of Se with other environmental contaminants are
993 between Se and Hg, where each may counteract the toxicity of the other (Cuvin-
994 Aralar and Furness, 1991) but also may increase bioaccumulation in tissues (e.g.,
995 Furness and Rainbow, 1990; Heinz and Hoffman, 1998). However, Se toxicity has
996 also been reported to be reduced by elevated levels of lead (Donaldson and
997 McGowan, 1989), copper and cadmium (Hill, 1974), silver (Jensen, 1975), and As
998 (Thapar et al., 1969; Stanley et al., 1994). Despite their common occurrence,
999 biological effects of metal contaminant mixtures are poorly understood and difficult
1000 to predict.

1001 Interactions between Se and vitamins A, C, and E, as well as sulfur-containing
1002 amino acids also have been documented (NAS-NRC, 1976, 1983; Kishchak, 1998;

1003 Eisler, 2000). The interactions may be synergistic or antagonistic in terms of effects
1004 on uptake and metabolism, and the degree of interaction is affected by numerous
1005 factors. Thus, the topic of interactions is too complex to be addressed in detail in this
1006 review, and only a few examples of recent studies are discussed. Nevertheless, some
1007 of the interactions of Se with other chemicals can be important factors in the design
1008 of field or laboratory studies and in the evaluation of results, and they should be
1009 taken into consideration.

1010 After adverse effects characteristic of Se toxicosis were observed in field studies at
1011 Kesterson Reservoir, California (described above), a series of laboratory studies was
1012 conducted, primarily with mallards, to help interpret the potential toxicity of
1013 different forms of Se, dietary sources of Se, and interactions with other dietary
1014 components including methionine, protein, and various trace elements that might be
1015 encountered in nature. Hamilton and Hoffman (2003) provide a review of the
1016 findings from the various laboratory studies, including Se concentrations in diets or
1017 tissues associated with the effects.

1018 Here we summarize only the laboratory studies conducted to assess interactions
1019 with As (Stanley et al., 1994), B (Stanley et al., 1996), and Hg (Heinz and Hoffman,
1020 1998) in addition to relevant field studies. Each of the laboratory studies involved
1021 varying levels of dietary exposures of breeding mallards to Se alone, one of the other
1022 elements alone, and Se in combination with the other chemical. In each study, Se
1023 and the other chemical caused significant adverse effects on reproduction when
1024 present alone in the diet at higher treatment levels, but the interactions varied by
1025 chemical. Antagonistic interactions between As and Se occurred whereby As
1026 reduced Se accumulation in duck livers and eggs, and reduced the effects of Se on
1027 hatching success and embryo deformities when dietary As concentrations were 100
1028 or 400 mg/kg. As the authors noted, however, the importance of the observed As-Se
1029 interaction in the environment is unknown because As may not be present in bird
1030 food items at contaminated sites in the form used in the study (sodium arsenate).

1031 There was little evidence of interaction between B and Se when ducks were fed the
1032 two chemicals in combination. When the diet contained 10 mg Se/kg plus 10 mg
1033 Hg/kg, the effects on reproduction were worse than for either Se or Hg alone, even
1034 though Se concentrations in eggs were elevated only modestly by the presence of
1035 Hg. The 10-mg Se/kg diet produced a mean of about 25 mg Se/kg on a dw basis (7.6
1036 mg Se/kg ww) in eggs, and reduced the hatching success of fertile eggs to 24.0%
1037 compared to 44.2% for controls. When 10 mg Hg/kg was fed along with the 10 mg
1038 Se/kg, Se concentrations in eggs rose only to about 31 mg/kg dw (9.3 mg/kg ww),
1039 but hatching success dropped to 1.4%. Either the embryotoxicity of the Se had been
1040 increased by the presence of Hg, the embryotoxicity of the Hg was added to that of
1041 the Se, or some combination of these synergistic effects had occurred. In any case,
1042 the 31 mg Se/kg measured in eggs was associated with a greater-than-expected level
1043 of embryonic death were one to focus only on the Se in the eggs. In addition to the
1044 number of young produced per female being significantly reduced in the above
1045 study, the frequency of teratogenic effects was significantly increased by the
1046 combination of Hg and Se in the diet, and Hg enhanced the storage of Se in duck
1047 tissues. Female mallards fed the combination diet had about 1.5 times higher hepatic
1048 Se concentrations than those fed the Se-only diet, and male mallards fed the
1049 combination diet had almost 12 times the Se concentration of those fed the Se-only
1050 diet. In contrast to the synergistic effects on reproduction, the combined Se plus Hg
1051 diet was less toxic to adult male mallards than either Se or Hg alone. In male
1052 mallards fed only the 10 mg Se/kg diet, livers contained a mean of about 32 mg
1053 Se/kg dw (9.6 mg Se/kg ww), but when 10 mg Hg/kg was also in the diet, male
1054 livers contained a mean of about 380 mg Se/kg (114 mg Se/kg ww). A value of 380
1055 mg Se/kg in the liver of ducks would almost certainly be equated with severe harm,
1056 but the coexistence of about 217 mg Hg/kg (65 mg Hg/kg ww) in the livers
1057 seemingly nullified the toxicity of the Se. Likewise, the 217 mg Hg/kg is well above
1058 the level normally associated with harm in birds; in this study a level of about 237
1059 mg Hg/kg (71 mg Hg/kg ww) was reported in the male mallards fed only the 10

1060 mg Hg/kg, and Hg-induced toxicity and mortality were observed in this group of
1061 males. Obviously, the Hg and Se had conferred a mutually antagonistic effect on
1062 each other, but only as far as the adult birds were concerned.

1063 Mercury and Se concentrations in the livers of various free-living carnivorous
1064 mammals often are highly correlated in a molar ratio of 1:1 (Scheuhammer, 1987;
1065 Furness and Rainbow, 1990; Cuvin-Aralar and Furness, 1991; Eisler, 2000). However,
1066 there is no consistent pattern for such a correlation in the livers of birds. For
1067 example, in diving ducks from San Francisco Bay, hepatic Hg and Se were
1068 correlated, but Se concentrations exceeded Hg concentrations by 6- to 15-fold on
1069 molar basis (Ohlendorf et al., 1986c, 1991). Elsewhere, Hg and Se concentrations
1070 were positively correlated in some bird livers, but not in others, or they were
1071 negatively correlated (see review by Ohlendorf, 1993). These relationships may
1072 change as birds remain at the sampling location (due to differential accumulation
1073 and loss rates for Hg and Se), they may vary because of differing relative
1074 concentrations of the two elements, and other factors (such as the chemical forms
1075 present) also may complicate the patterns of bioaccumulation.

1076 When there is a low concentration of Hg, a lower molar ratio is observed; however,
1077 at high Hg and Se concentrations in the liver, most Se binds Hg resulting in a Hg:Se
1078 ratio greater than 1.0 (Kim et al. 1996). For example, livers of black-footed albatross
1079 that contained total mercury (THg) concentrations over 100 mg/kg had an
1080 equivalent molar ratio of 1:1 between THg and Se, but such a relationship was
1081 unclear when birds had relatively low Hg levels. Studies by Henny et al. (2002) and
1082 Spalding et al. (2000) have shown high correlations of Se with IoHg on a molar basis
1083 in livers of fish-eating birds. As the THg concentration increased, the percentage
1084 present as methylmercury (MeHg) decreased. Those authors suggested that Se may
1085 contribute to the sequestration of IoHg, thereby reducing its toxicity. This conclusion
1086 would be consistent with the results of a Se-Hg interaction study with mallards by
1087 Heinz and Hoffman (1998) described above.

1088 Recent work by Eagles-Smith et al. (2009) provides a useful understanding of Se-Hg
1089 relationships. They assessed the role of Se in demethylation of MeHg in the livers of
1090 adults and chicks of four waterbird species that commonly breed in San Francisco
1091 Bay (American avocets, black-necked stilts, Caspian terns [*Hydroprogne caspia*;
1092 formerly *Sterna caspia*], and Forster's terns [*Sterna forsteri*]). In adults (all species
1093 combined) there was strong evidence for a threshold model where demethylation of
1094 MeHg occurred above a hepatic THg concentration threshold of 8.51 ± 0.93 mg/kg,
1095 and there was a strong decline in percent MeHg values as THg concentrations
1096 increased above 8.51 mg/kg. Conversely, there was no evidence for a demethylation
1097 threshold in chicks, and they found that percent MeHg values declined linearly with
1098 increasing THg concentrations. For adults, they also found taxonomic differences in
1099 the demethylation responses, with avocets and stilts showing a higher
1100 demethylation rate than terns when concentrations exceeded the threshold, whereas
1101 terns had a lower demethylation threshold (7.48 ± 1.48 mg/kg) than avocets and
1102 stilts (9.91 ± 1.29 mg/kg). Selenium concentrations were positively correlated with
1103 IoHg in livers of birds above the demethylation threshold, but not below, suggesting
1104 that Se may act as a binding site for demethylated Hg and may reduce the potential
1105 for secondary toxicity.

1106 Similar findings were reported by Scheuhammer et al. (2008) for common loons
1107 (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*), although the thresholds were
1108 very different. In liver, both species had a wide range of THg concentrations,
1109 substantial demethylation of MeHg, and co-accumulation of Hg and Se. There were
1110 molar excesses of Se over Hg up to about 50-60 mg Hg/kg, above which there was
1111 an approximate 1:1 molar ratio of Hg:Se in both species. Thus, the amount of Se bound
1112 to Hg at any given concentration of THg is likely to vary among species, suggesting that the
1113 8.5 mg Hg/kg threshold described above is not a universal one.

1114 At this time it is not possible to enumerate what concentrations of Se need to be in
1115 eggs or tissues to cause harm when certain concentrations of other contaminants

1116 such as Hg are also present in the samples. Likewise, the concentrations of
1117 combinations of Se and other chemicals that would lead one to conclude that no
1118 harm from Se, or the other chemical, is likely to occur are unknown. However, when
1119 elevated concentrations of other contaminants, especially Hg, are found along with
1120 Se in eggs or tissues, caution should be exercised in interpreting the significance of
1121 the Se (and the other contaminant). When warranted and feasible (due to time and
1122 resource constraints), this caution would translate into conducting careful field
1123 studies at the contaminated site to determine if reproduction and adult health are
1124 normal, compared to an uncontaminated reference area.

1125 **Hormesis**

1126 Selenium is an essential trace element for bird diets, as described above, and
1127 inadequate dietary levels of bioavailable Se may result in low Se in eggs. When
1128 poultry diets contain Se concentrations of less than 0.30 mg/kg and eggs contain less
1129 than about 0.66 mg/kg dw (0.20 mg/kg ww), they are considered to be below the
1130 “adequate” range (Puls, 1988).

1131 Consideration of the hormetic effects of Se may result in lowering of thresholds for
1132 diet and eggs described above. A recent paper by Beckon et al. (2008) used the mean
1133 response data for the control and five treatment levels from the mallard study by
1134 Heinz et al. (1989) to evaluate potential hormetic effects exhibited by the treatment
1135 groups. They concluded that the EC₁₀ from that study was 7.7 mg Se/kg (although
1136 their Figure 5 says 7.3 mg Se/kg). Because Se concentrations in bird eggs may be
1137 used in setting site-specific water quality standards for Se (e.g., Great Salt Lake; State
1138 of Utah, 2008), the difference in conclusions between the Ohlendorf (2003) and
1139 Beckon et al. (2008) results are important from a regulatory as well as scientific
1140 standpoint. Consequently, further analyses of the available data from the six studies
1141 with mallards (Heinz et al., 1987, 1989; Heinz and Hoffman, 1996, 1998; Stanley et
1142 al., 1994, 1996) are underway by the authors of this chapter.

1143 **Conclusions and Recommendations**

1144 Selenium is an essential nutrient for birds, with a narrow range of concentrations
1145 between what is a beneficial diet (< 3 mg/kg dw) and what represents a threshold
1146 for reproductive impairment (in the range of 3 to 8 mg/kg, depending on species
1147 and the form of Se in the diet). When birds eat a high-Se diet, Se levels in the diet are
1148 quickly reflected in concentrations in eggs, liver, and blood, but more slowly in
1149 muscle. Similarly, when birds are switched from a high-Se diet to one with a lower
1150 concentration (or when they migrate from a high-Se area to a Se-normal area), the
1151 eggs, liver, and blood adjust relatively quickly to the lower concentrations.

1152 Kidneys are not as useful as livers for diagnosing Se status of birds, although the
1153 concentrations in kidneys and livers are highly correlated. In Se-normal areas,
1154 concentrations in kidneys tend to be higher than those in the liver, but
1155 concentrations in the two tissues are similar in birds from high-Se areas. Feathers
1156 can be useful under some circumstances, but it is important to recognize that Se
1157 concentrations in feathers reflect the exposure of the bird when the feathers were
1158 developing, not their current exposure.

1159 Background, elevated, and various effect levels of Se in bird diets, eggs, and various
1160 tissues are summarized in Table 1. We present there a range of effect concentrations
1161 because different techniques have been used to develop them, and the reader can
1162 select from the range of values those that are appropriate for the degree of
1163 protectiveness (conservatism) desired under a particular set of circumstances.

1164 Based on our experience and review of the literature, we recommend the values
1165 presented in Table 2 as diagnostic levels for Se concentrations in eggs, livers, and
1166 diet to evaluate the probability that Se may be causing adverse effects in birds. Se
1167 concentrations in eggs and livers should be considered the primary diagnostic
1168 levels, complemented by Se levels in the diet and observed effects on egg
1169 hatchability or signs of toxicosis such as those described for liver or other tissues. As
1170 stated previously, when Se concentrations are known for both the eggs and livers of

1171 breeding females, judgments on the hazards of Se to reproduction should be based
1172 on Se in the egg.

1173 Short of doing a time-consuming study of reproductive success, analysis of eggs is
1174 by far the best way to determine status of a population with respect to potential
1175 reproductive impairment. No single criterion is available for diagnosis of Se
1176 toxicosis in young or adult birds, but Se toxicosis is indicated when elevated Se
1177 concentrations in tissues (especially when greater than 20 mg/kg in the liver) are
1178 accompanied by emaciation, poor quality of shed nails, bilaterally symmetrical
1179 alopecia of the head and neck, toxic hepatic lesions, and necrosis of maxillary nails.

1180 Regardless of which kind of sample is being analyzed (diet, egg, or other tissue), we
1181 highly recommend measuring moisture content of the samples and reporting those
1182 values along with the Se concentration. The literature contains a mixture of wet-
1183 weight and dry-weight concentrations in different media, and it is difficult to relate
1184 concentrations on one basis to the other without knowing the moisture content of
1185 the samples. This is important because moisture content varies by sample type and
1186 handling procedures.

1187 Physiological changes associated with Se exposure in field-collected or laboratory-
1188 exposed birds generally involve changes in measurements associated with liver
1189 pathology and glutathione metabolism (e.g., glycogen, protein, total sulfhydryl and
1190 protein-bound sulfhydryl, concentrations; glutathione peroxidase activity). As
1191 dietary and tissue concentrations of Se increase, increases in plasma and hepatic
1192 glutathione peroxidase activities occur, followed by dose-dependent increases in the
1193 ratio of hepatic oxidized to reduced glutathione, and ultimately hepatic lipid
1194 peroxidation. At a given tissue (or egg) Se concentration, one or more of these
1195 oxidative effects were associated with teratogenesis (when Se concentrations in eggs
1196 reached about 15 mg/kg dw = 4.6 mg/kg ww), reduced growth of ducklings (at
1197 about 50 mg Se/kg dw = 15 mg Se/kg ww in liver), diminished immune system (at

1198 about 16 mg Se/kg dw = 5 mg Se/kg ww in liver) and histopathological lesions
1199 (about 96 mg Se/kg dw = 29 mg Se/kg ww in liver) in adults.

1200 The characteristic reproductive effects of Se observed in both field and laboratory
1201 studies include reduced hatchability of eggs (due to embryo mortality) and high
1202 incidence of developmental abnormalities (due to teratogenesis). Selenium-induced
1203 abnormalities are often multiple and include defects of the eyes (microphthalmia
1204 and possible anophthalmia [i.e., abnormally small or missing eyes]), feet or legs
1205 (amelia and ectrodactylia [absence of legs or toes]), beak (incomplete development
1206 of the lower beak, spatulate narrowing of the upper beak), brain (hydrocephaly and
1207 exencephaly [fluid accumulation in the brain and exposure of the brain]), and
1208 abdomen (gastroschisis [an open fissure of the abdomen]).

1209 Selenium interacts with a number of other environmental contaminants and
1210 nutrients of interest for birds. The interactions of Se with Hg have been studied most
1211 extensively, but interactions with As also may be important. Selenium and Hg each
1212 may counteract or increase the toxicity of the other but also may increase
1213 bioaccumulation in tissues. Dietary Hg and Se together were more harmful to
1214 mallard reproduction than either element was alone, while they were less toxic to
1215 adult birds in combination than they were alone. Consequently, where Hg may be
1216 elevated, both Se and Hg should be evaluated. In a similar study of Se and inorganic
1217 As, interactions between As and Se were antagonistic, whereby As reduced Se
1218 accumulation in duck livers and eggs, and reduced the effects of Se on hatching
1219 success and embryo deformities.

1220 Recent work on Se-Hg interactions has shown strong evidence for a threshold above
1221 which demethylation of MeHg occurred, and there was a strong decline in percent
1222 MeHg values as THg concentrations increased above the threshold. Conversely,
1223 there was no evidence for a demethylation threshold in chicks, and percent MeHg
1224 values declined linearly with increasing THg concentrations. For adults, there were
1225 taxonomic differences in the demethylation responses, with avocets and stilts

1226 showing a higher demethylation rate than terns when concentrations exceeded the
1227 threshold, whereas terns had a lower demethylation threshold than avocets and
1228 stilts. Selenium concentrations were positively correlated with IoHg in livers of birds
1229 above the demethylation threshold, but not below, suggesting that Se may act as a
1230 binding site for demethylated Hg and may reduce the potential for secondary
1231 toxicity.

1232 In summary, the ecotoxicology of Se is complex, because of the variable chemical
1233 forms in which it occurs in the environment, its interactions with other
1234 environmental contaminants, and large differences in species sensitivity to the
1235 adverse effects of Se. The most likely effects to be observed in the field are
1236 reproductive impairment, which has been documented at a number of locations
1237 during the past 25 years or so. However, Se toxicosis and mortality of adult birds
1238 also has been observed and may occur when exposures are higher than those
1239 causing reproductive impairment. The assessment values for diet, eggs, and other
1240 tissues presented in Table 1 can be used to evaluate risks of adverse effects in birds.

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- 1597 Yasumoto, K., T. Suzuki, and M. Yoshido. 1988. Identification of selenomethionine in
1598 soybean protein. *J. Agric. Food Chem.* 36:463-467.

1599 Table 1. Published assessment values for effects of dietary or tissue concentrations of Se on birds.

Medium and Level/Status ^a	Concentration (mg Se/kg, dw)	Effects	Comments	References
Diet^b				
Adequate	0.30-1.1	Nutritional needs are met for poultry	Lower dietary concentrations are marginal or deficient, and diets must be fortified	Puls, 1988
High	3.0-5.0	Levels are excessive but not considered toxic to poultry	Poultry are relatively sensitive to effects of selenium	Puls, 1988
Toxic	>5.0	Reduced egg hatchability and teratogenic effects in embryos/chicks	Poultry are relatively sensitive to effects of selenium	Puls, 1988
Background	<3.0	None	Deficiencies associated with lower concentrations have not been reported in wild birds	USDI, 1998; Eisler, 2000
Reproductive impairment	3-8	Reduced egg hatchability; potential deformities in embryos/chicks at upper end of range	Sensitivity varies by species	USDI, 1998; Eisler, 2000

1600

Reproductive impairment	4.0 (95% CI = <0.5-7.3)	EC ₁₀ for reduced egg hatchability	Based on studies of mallard, American kestrel, chicken, black-crowned night-heron, eastern screech-owl and ring-necked pheasant using logistic regression analysis	Wayland et al. (2007)
Reproductive impairment	4.4 (95% CI = 3.8-4.8)	EC ₁₀ for reduced egg hatchability	Based on results of six laboratory studies with mallards, using hockey-stick regression analysis	Adams (pers. comm.; see Ohlendorf 2007)
Reproductive impairment	4.9 (95% CI = 3.6-5.7)	EC ₁₀ for reduced egg hatchability	Based on results of six laboratory studies with mallards, using logistic regression analysis	Ohlendorf, 2003
Eggs^c				
Adequate	0.66-5.0 (0.20-1.5 ww)	Nutritional needs are met for poultry	Lower dietary concentrations are marginal or deficient, and diets must be fortified	Puls, 1988

1601

High	5.0-16 (1.5-5.0 ww)	Levels are excessive and upper end of range may be toxic to poultry	Poultry are relatively sensitive to effects of selenium	Puls, 1988
Toxic	>8.2 (>2.5 ww)	Reduced egg hatchability and teratogenic effects in embryos/chicks	Poultry are relatively sensitive to effects of selenium	Puls, 1988
Background	Mean < 3.0 (typically 1.5-2.5); individual eggs <5	None	Concentrations may be higher in some marine birds (Ohlendorf and Harrison, 1986; Braune et al., 2002)	Ohlendorf and Harrison, 1986; Skorupa and Ohlendorf, 1991; USDI, 1998; Eisler, 2000
Reproductive impairment	6-7 (about 1.8-2.1 ww)	EC ₁₀ on a clutch-wise (or hen-wise) basis and EC ₀₃ on egg-wise basis	Based on results of extensive field studies of black-necked stilts	Skorupa, 1998b, 1999
Reproductive impairment	7.7 (about 2.3 ww)	EC ₁₀ for reduced egg hatchability	Based on results of one laboratory study with mallards, assuming hormetic effects	Beckon et al., 2008
Reproductive impairment	9.0	EC _{8.2} for impaired clutch viability	Based on results of one laboratory study with mallards, using linear regression analysis	Lam et al., 2005

1602

Reproductive impairment	12 (95% CI = 6.4-16)	EC ₁₀ for reduced egg hatchability	Based on results of six laboratory studies with mallards, using logistic regression analysis	Ohlendorf, 2003
Reproductive impairment	12 (95% CI = 9.7-14)	EC ₁₀ for reduced egg hatchability	Based on results of six laboratory studies with mallards, using hockey stick analysis	Adams (pers. comm.; see Ohlendorf 2007)
Reproductive impairment	14	EC _{11.8} for reduced egg hatchability	Based on results of extensive field studies of black-necked stilts	Lam et al., 2005
Teratogenicity	13-24	Threshold for teratogenic effects on population level	Sensitivity varies widely by species	Skorupa and Ohlendorf, 1991
Teratogenicity	23	EC ₁₀ for teratogenic effects in mallard	Mallard is considered a "sensitive" species	Skorupa, 1998b; USDI, 1998
Teratogenicity	37	EC ₁₀ for teratogenic effects in stilt	Stilt is considered an "average" species	Skorupa, 1998b; USDI, 1998
Teratogenicity	74	EC ₁₀ for teratogenic effects in American avocet	Avocet is considered a "tolerant" species	Skorupa, 1998b; USDI, 1998
Liver^d				
Adequate	1.2-3.3 (0.35-1.0 ww)	Nutritional needs are met	Lower liver concentrations are marginal or deficient, and diets must be fortified	Puls, 1988

High	6.6-20 (2.0-6.0 ww)	Levels are excessive but not considered toxic to poultry	Poultry are relatively sensitive to effects of selenium	Puls, 1988
Toxic	13-76 (4.0-23 ww)	Reduced egg hatchability and teratogenic effects in embryos/chicks	Poultry are relatively sensitive to effects of selenium	Puls, 1988
Background for freshwater and terrestrial species	<10	None	Deficiencies associated with lower concentrations have not been documented in wild birds	USDI, 1998; Eisler, 2000
Background for marine species	20 to 75 in some species (see text)	None	Found in livers of several species from uncontaminated areas	Elliott et al., 1992; Dietz et al., 1996; Trust et al., 2000; Grand et al., 2002; Mallory et al., 2004; Campbell et al., 2005; Elliott, 2005
Elevated and potentially toxic	10-20	Considered suspicious of selenium toxicosis when accompanied by symptoms listed for toxic effects	Sensitivity varies by species	Ohlendorf et al., 1988; Albers et al., 1996; O'Toole and Raisbeck, 1997, 1998
Toxic	20-25	Diagnostic when accompanied by emaciation, poor quality of shed nails, bilaterally symmetrical alopecia of the head and neck, hepatic lesions, and necrosis of maxillary nails	Based on field observations and laboratory studies with mallards	Ohlendorf et al., 1988; Albers et al., 1996; O'Toole and Raisbeck, 1997, 1998

1603

Toxic	351-735	Many effects on liver and other tissues	Common eiders seem to be more tolerant of selenium in tissues than are mallards	Franson et al., 2007
Kidney^e				
Adequate	2.2-5.2 (0.50-1.2 ww)	Nutritional needs are met in poultry	Similar to wild birds, concentrations tend to be higher than in liver	Moksnes, 1983; Puls, 1988
High	6.4-22 (1.5-5.2 ww)	Levels are excessive but not considered toxic to poultry	Similar to wild birds, concentrations tend to be equal to or lower than in liver	Moksnes, 1983; Puls, 1988
Muscle^f				
Adequate	0.49-4.9 (0.13-1.3 ww)	Nutritional needs are met	Lower muscle concentrations are marginal or deficient, and diets must be fortified	Puls, 1988
High	1.5-21 (0.40-5.5 ww)	Levels are excessive but may not be toxic to poultry	Wide range of concentrations that overlaps with toxic level	Puls, 1988

1604

Toxic	4.9 (1.3 ww)	Toxic level is below the midpoint of the "high" range	Concentrations in muscle are not very useful for diagnosing current exposure because of long lag in reaching equilibrium	Puls, 1988
Background	1-3	None	Accumulation in muscle varies by bird species and chemical form of selenium; concentrations above background in muscle more useful for assessing human health risks than diagnosing toxic effects in birds	USDI, 1998; Eisler, 2000
Bloods				
Adequate	0.62-0.96 0.13-0.20 ww	Nutritional needs are met	Lower blood concentrations are marginal or deficient, and diets must be fortified	Puls, 1988
Background	0.48-1.9 (0.10-0.40 ww)	None	Deficiencies associated with lower concentrations have not been documented in wild birds	USDI, 1998; Eisler, 2000

Provisional threshold warranting further study	4.8 (1.0 ww)	Interpretive relationship to effects is limited, but elevated levels associated with effects on reproduction or survival	Blood selenium concentrations are good indicator of current/recent exposure, and especially important for sampling when animals should not be sacrificed	Heinz et al., 1990; Heinz and Fitzgerald, 1993a; O'Toole and Raisbeck, 1997; USDI, 1998; Yamamoto et al., 1998; Santolo et al., 1999; Eisler, 2000
Feathers^h				
Background	1-4 (typically 1-2)	None	Based on breast feathers; concentrations in feathers vary by type and reflect exposure at the time feathers were grown, rather than current exposure	Burger, 1993; Ohlendorf, 1993; USDI, 1998; Eisler, 2000
Provisional threshold warranting further study	5	Interpretive relationship to effects is limited, but elevated levels associated with exposure when the feathers were developing	Feather selenium concentrations are not good indicator of current/recent exposure, but may be useful if limitations are understood (see text)	Burger, 1993; Ohlendorf, 1993; USDI, 1998; Eisler, 2000

1605 ^aTypical moisture content (%) and approximate conversion factor are shown in footnotes for each medium. Values that are
 1606 shaded are based on domestic poultry rather than wild species.

1607 ^bVariable moisture; laboratory diet typically ~10%, but natural diet varies widely (<10 to >90%)

- 1608 ^c65-80% moisture, varying with species and incubation stage; use 70% (i.e., factor of 3.3) for approximate conversion
- 1609 ^d70% moisture; use factor of 3.3 for approximate conversion
- 1610 ^e76-78% moisture, based on limited data; use factor of 4.3 for approximate conversion
- 1611 ^f74% moisture; use factor of 3.8 for approximate conversion
- 1612 ^g79% moisture in lab studies, variable under field conditions; use factor of 4.8 for approximate conversion
- 1613 ^h10% moisture assumed (not well defined); use factor of 1.1 for approximate conversion

1614 Table 2. Recommended assessment values for effects of dietary or tissue concentrations of Se
 1615 on birds.

Medium and Level/Status/Effects ^a	Concentration (mg Se/kg, dw)	Comments
Diet^b		
Background	<3.0	Typical concentrations in diet items for birds; deficiencies associated with low concentrations have not been reported in wild birds
Reproductive impairment	<4.0	Low probability for reduced egg hatchability; value based on studies of multiple species
Reproductive impairment	>5.0	Elevated probability for reduced egg hatchability in sensitive species; effects down to this concentration may be measurable in the laboratory but unlikely to be detectable in the field unless dietary concentrations are considerably higher
Eggs^c		
Background	Mean < 3.0 (typically 1.5-2.5); individual eggs <5	Concentrations may be higher in some marine birds
Reproductive impairment	<8.0	Low probability for reduced egg hatchability, including effects in sensitive species
Reproductive impairment	>12	Elevated probability for reduced egg hatchability in sensitive and moderately sensitive species
Teratogenicity	<20	Low probability for teratogenic effects in most species, and threshold for statistically discernable incidence in sensitive species such as mallard
Teratogenicity	>35	Probability for teratogenic effects in species of "average" sensitivity such as black-necked stilt
Liver^d		
Background for freshwater and terrestrial species	<10	Low probability of adverse effects in these species

Background for some marine species	20 to 75 in some species (see text)	Low probability of adverse effects in these species; must consider species differences compared to freshwater and terrestrial species
Elevated and potentially toxic in freshwater and terrestrial species	10-20	Considered suspicious of selenium toxicosis when accompanied by symptoms listed for toxic effects (see text); sensitivity varies by species
Toxic	>20	Diagnostic of Se toxicosis when accompanied by emaciation, poor quality (and sloughing) of nails, bilaterally symmetrical alopecia of the head and neck, hepatic lesions, and necrosis of maxillary nails; based on field observations and laboratory studies with mallards

1616

1617 Notes: No specific recommendations are made for kidney, muscle, blood, or feathers,
 1618 although each of them can indicate levels of exposure. Kidney concentration is generally
 1619 correlated with liver; muscle responds more slowly than eggs, liver, or blood in reflecting
 1620 current exposure; and feathers reflect exposure at the time they were growing rather than
 1621 the time of sampling.

1622 ^aTypical moisture content (%) and approximate conversion factor are shown in footnotes
 1623 for each medium.

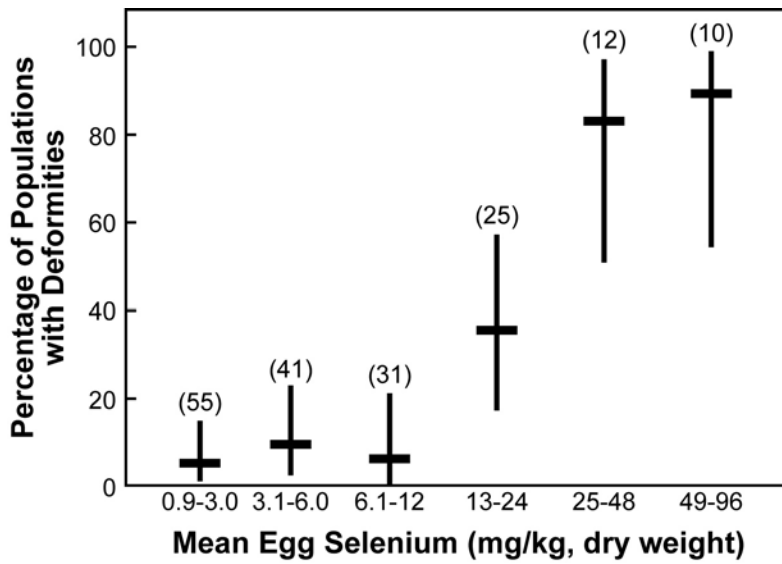
1624 ^bVariable moisture; laboratory diet typically ~10%, but natural diet varies widely (<10 to
 1625 >90%)

1626 ^c65-80% moisture, varying with species and incubation stage; use 70% (i.e., factor of 3.3)
 1627 for approximate conversion

1628 ^d70% moisture; use factor of 3.3 for approximate conversion

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1630



1631

1632 Figure 1. Dose-response relation between mean egg Se and teratogenic classification of
 1633 aquatic bird populations (from Skorupa and Ohlendorf, 1991). For each dose interval, the
 1634 observed percentage of populations classified as teratogenic is plotted along with 95%
 1635 binomial confidence intervals. Sample sizes (number of populations assessed) for each dose
 1636 interval are listed above the response plots.

1637