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Effects of Dietary Selenium on Tissue Concentrations, Pathology, Oxidative Stress, and Immune Function in Common Eiders (*Somateria mollissima*)

J. Christian Franson
USGS National Wildlife Health Center, Madison, Wisconsin, USA

David J. Hoffman, Alicia Wells-Berlin, and Matthew C. Perry
USGS Patuxent Wildlife Research Center, Laurel, Maryland, USA

Valerie Shearn-Bochsler and Daniel L. Finley
USGS National Wildlife Health Center, Madison, Wisconsin, USA

Paul L. Flint
USGS Alaska Science Center, Anchorage, Alaska, USA

Tuula Hollmén
Alaska SeaLife Center and School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Seward, Alaska, USA

Common eiders (*Somateria mollissima*) were fed added Se (as L-selenomethionine) in concentrations increasing from 10 to 80 ppm in a pilot study (Study 1) or 20 (low exposure) and up to 60 (high exposure) ppm Se in Study 2. Body weights of Study 1 ducks and high-exposure ducks in Study 2 declined rapidly. Mean concentrations of Se in blood reached 32.4 ppm wet weight in Study 1 and 17.5 ppm wet weight in high-exposure birds in Study 2. Mean Se concentrations in liver ranged from 351 (low exposure, Study 2) to 1252 ppm dry weight (Study 1). Oxidative stress was evidenced by Se-associated effects on glutathione metabolism. As Se concentrations in liver increased, Se-dependent glutathione peroxidase activity, glutathione reductase activity, oxidized glutathione levels, and the ratio of hepatic oxidized to reduced glutathione increased. In Study 2, the T-cell-mediated immune response was adversely affected in high-exposure eiders, but ducks in the low-exposure group exhibited evidence of an enhanced antibody-mediated immune response. Gross lesions in high-exposure ducks included emaciation, absence of thymus, and loss of nails from digits. Histologic lesions included severe depletion of lymphoid organs, hepatopathy, and necrosis of feather pulp and feather epithelium. Field studies showed that apparently healthy sea ducks generally have higher levels of Se in liver than healthy fresh-water birds, but lower than concentrations found in our study. Data indicate that common eiders and probably other sea ducks possess a higher threshold, or adverse effect level, for Se in tissues than fresh-water species. However, common eiders developed signs of Se toxicity similar to those seen in fresh-water birds.

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Address correspondence to Dr. J. Christian Franson, USGS National Wildlife Health Center, Madison, WI 53711, USA. E-mail: chris_franson@usgs.gov

Selenium is a natural component of the earth's crust and becomes environmentally available from weathering of rock and a variety of anthropogenic activities, including burning of fossil fuels, irrigation of highly seleniferous soils, mining, and smelting (Eisler, 2000). Although Se is an essential trace element for birds and other animals, the margin between required and toxic dietary levels is relatively small, and it readily bioaccumulates in food chains to levels that are poisonous to wildlife (Ohlendorf, 1996; Eisler, 2000). In the early 1980s, Se from agricultural drainwater was identified as the cause of mortality and reproductive problems in birds in central California, and additional field cases of Se poisoning in birds were described in other nonmarine environments (Ohlendorf et al., 1988; Skorupa, 1998).

Many of the field and laboratory studies of Se exposure in birds were conducted in fresh-water species and demonstrated a variety of adverse effects, including altered glutathione

metabolism and oxidative stress, teratogenesis, reduced growth of young, impaired immune response, and histopathologic lesions (Hoffman, 2002). Sublethal effects may occur in birds when Se concentrations in the liver reach 10 ppm wet weight (ww) (equivalent to approximately 33 ppm dry weight [dw]), and death may occur at 20 ppm ww (about 66 ppm dry weight; Heinz, 1996). Less is known about the relationship between Se concentrations in the blood and adverse effects in birds, but as experimental mallards (*Anas platyrhynchos*) began to die due to Se exposure, mean concentrations of Se in the blood of survivors were 5 to 14 ppm ww (approximately 20 to 70 ppm dw) (Heinz & Fitzgerald, 1993). In another study, mallards fed 60 ppm Se contained 16 ppm ww Se in their blood after approximately 40 d (O'Toole & Raisbeck, 1997). Selenium concentrations in the blood and liver of healthy wild fresh-water birds and controls in experimental studies are normally less than 0.4 and 2 ppm ww, respectively (or about 2 and 6 ppm dw) (Heinz & Fitzgerald, 1993; O'Toole & Raisbeck, 1997; U.S. Department of the Interior, 1998).

Marine waterfowl in arctic and subarctic regions tend to accumulate concentrations of Se in their tissues that would be considered elevated or even toxic in fresh-water birds. In a study of 4 eider species in Alaska, mean Se concentrations in liver ranged from a low of 7.85 ppm dw in common eider (*Somateria mollissima*) females to 124 ppm dw in spectacled eider (*Somateria fischeri*) males (Stout et al., 2002). White-winged scoters (*Melanitta fusca*), black scoters (*Melanitta nigra*), and spectacled eiders contained maximum Se concentrations in their livers of 53, 32, and 77 ppm dw, respectively (Henny et al., 1995). On nesting grounds in Alaska, Grand et al. (2002) found blood Se concentrations of 19.3 ppm ww in male spectacled eiders, 12.8 ppm ww in incubating female spectacled eiders, and 7.29 ppm ww in common eiders sampled after hatch. Sympatric emperor geese (*Chen canagica*) nesting in the same area as the spectacled eiders had mean Se concentrations of up to 5.6 ppm ww in their blood (Franson et al., 1999). On the arctic coastal plain and the Beaufort Sea coast of northern Alaska, mean Se concentrations in blood of adult sea ducks ranged from 8.68 to 10.2 ppm ww in king eiders (*Somateria spectabilis*), 2.92 to 14.7 ppm ww in spectacled eiders, 36.1 ppm dw (approximately 7.36 ppm ww) in common eiders, and 48.8 ppm dw (approximately 11.6 ppm ww) in long-tailed ducks (*Clangula hyemalis*) (Franson et al., 2004; Wilson et al., 2004). Selenium levels in livers of common eiders and king eiders in the Canadian arctic ranged from 10.2 to 35.6 ppm dw (Wayland et al., 2001). Male common eiders in Finland contained mean Se concentrations of up to 22.5 and 47 ppm dw in liver, and incubating females had mean Se concentrations in blood of up to 2.86 ppm ww (Hollmén et al., 1998; Franson et al., 2000a, 2000b).

Recent field studies evaluated biomarker responses in relation to concentrations of trace elements, including Se, in marine waterfowl. Nesting emperor geese showed early signs of oxidative stress with increasing Se concentrations in the blood, but studies with common eiders and spectacled eiders indicated no adverse health effects of Se exposure (Trust et al., 2000; Franson et al., 2002; Wayland et al., 2002, 2003). In the present study, experiments evaluated the effects of dietary Se exposure on tissue concentrations, pathology, oxidative stress, and immune function in the common eider as a representative marine waterfowl species and surrogate for the threatened spectacled eider and Steller's eider (*Polysticta stelleri*). Findings were compared with results from previous studies in mallards.

MATERIALS AND METHODS

Ducks and Se Exposure, Study 1

To evaluate the response of common eiders to dietary Se, a preliminary range-finding study was conducted at the National Wildlife Health Center, Madison, WI, beginning in February 2003. Three common eiders purchased from a game breeder were housed and fed as described by Hollmén et al. (2003) in a 30-cm pool of fresh water equipped with a swimming-pool filter (Dayton Electric, Niles, IL). After a 3-wk acclimation period, Se was added to Mazuri Sea Duck Diet (number 5681, PMI Nutrition International, Brentwood, MO; 21.5% protein, 0.65 ppm Se as sodium selenite) in increasing concentrations (beginning with 10 ppm on d 0, 20 on d 14, 40 on d 28, 60 on d 56, and 80 on d 70). Selenium, as L-selenomethionine (Fisher Scientific, Chicago, IL), was dissolved in tap water (2% of feed by weight), sprayed on feed in a metal tray, and then mixed by rotating in a plastic drum. Water (2% by weight) also was added to the feed of control ducks. Four to six subsamples (approximately 20 g each) of each feed mix were collected for Se analysis. Blood samples were collected before the initiation of treated diets, weekly for the first 2 wk, then biweekly for the remainder of the study. Ducks were euthanized by CO_2 inhalation when they had lost approximately 30% of body weight, which occurred after 60–78 d on Se diets.

Ducks and Se Exposure, Study 2

Common eider eggs were collected from a nesting area near Islesboro, ME, in May 2004, transported to the USGS Patuxent Wildlife Research Center, Laurel, MD, and placed in an incubator (G.Q.F. Manufacturing, Inc., model 1502) at 37.5°C and 59% relative humidity until pipped. After pipping, eggs were transferred to a hatcher (G.Q.F. Manufacturing, Inc., model 1550) at 37.5°C and 70% relative humidity until hatch. As the ducklings grew, they were provided access to increasingly deeper water. When ducklings were fully feathered, at 1 mo of age, they were moved to 11.5-m^2 pens with gravel substrate. Each pen had a conical rubber-lined pool (2.1 m diameter, 70–80 cm deep at the center) with constantly flowing fresh water, and ducks had access to salt water (salinity = 35 ppt) prepared by mixing sea salt (Instant Ocean, Aquarium Systems, Mentor, OH) with fresh water in a stainless steel bowl. All ducklings were fed Mazuri Duckling Starter Diet (number

5641, PMI Nutrition International, Brentwood, MO; 20% protein, 0.53 ppm Se as sodium selenite) ad libitum until they were 1 mo of age, after which they were fed Mazuri Sea Duck Diet ad libitum.

When eiders were approximately 6 mo old, they were divided into 3 groups, when preexposure weights were recorded, and whole blood and plasma samples were collected from all ducks. Based on the results of Study 1, it was suspected that a diet of 60 ppm Se or more would cause substantial weight loss in eiders. A low-exposure (20 ppm) and a high-exposure (60 ppm Se) treatment were selected for Study 2. Six ducks (3M:3F) were fed control diet with no added Se (1 control duck died of undiagnosed causes on d 36 and all data from this bird were excluded from the study), 7 ducks (4M:3F) were fed 20 ppm added Se, and 6 ducks (3M:3F) were fed 60 ppm added Se. Feed and mixing procedures were as in Study 1, except that dissolved L-selenomethionine was sprayed onto the feed in an electric mixer (Hobart, Troy, OH). Because the birds initially avoided the 60-ppm diet, they were placed on untreated feed after 4 d. Treated feed was resumed 26 d later, but in an effort to accustom the birds to the 60-ppm diet, they were fed 20 ppm Se for wk 1, 40 ppm Se for wk 2, and then 60 ppm Se for the remainder of the study. For the timeline of the study, d 0 is defined as the day that treated feed was resumed. Whole blood and plasma were collected from all ducks on d 7, 13, 20, and biweekly thereafter. One duck in the high-exposure group died on d 30 and 1 duck was euthanized (CO_2 inhalation) on d 32 because it developed a staggering gait and had lost 35% of its body weight. Surviving ducks in the high-exposure group were euthanized on d 35, after they had lost approximately 30% of their body mass, and control and low-exposure groups were euthanized on d 83.

Immune Function Tests

Eiders in Study 2 were tested for antibody-mediated immunity using sheep red blood cells (SRBC) and for T-cell-mediated immunity with the phytohemagglutinin (PHA) skin test (Fairbrother et al., 2004; Wayland et al., 2002). On d 29, eiders were inoculated intravenously with 1 ml/kg body weight of 10% SRBC in phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO), and 6 d later, plasma was collected for SRBC hemagglutination tests. Plasma for SRBC antibody testing was also collected from control and low-exposure groups when they were euthanized, 54 d after SRBC inoculation. Fifty microliters of PBS was added to each U-shaped well of 96-well plastic microtiter plates (Corning, Corning, NY). After heating at 56°C for 30 min, 50 µl plasma was added in duplicate across the first row of microtiter wells. Serial twofold dilutions were carried out by transferring 50 µl from well to well. The last 50 µl was discarded and the highest dilution was 1/256. Fifty microliters of 0.5% SRBC in PBS was added to each well and plates were incubated, covered, at 37°C for 3 h. Antibody titers are expressed as the reciprocal of the highest dilution in which agglutination occurred. On d 29, the thickness of the right wing web was measured to the nearest 0.01 mm with a pressure-sensitive micrometer (Dyer, Lancaster, PA), and 0.1 ml PHA-P (Sigma-Aldrich, St. Louis, MO) in PBS (1 mg/ml) was injected intradermally. The wing web was measured 24 h later and the response to PHA-P was calculated as the difference between the pre- and postinjection measurements (Smits et al., 1999).

Plasma and Tissue Biochemistries, Serum Protein Electrophoresis

Many of the biochemical measurements selected have been used to indicate selenium and mercury toxicity in birds, or are known to reflect organ damage and related physiological disturbances. Plasma enzyme activities were measured on a centrifugal analyzer (Centrifichem 500; Baker Instrument Corporation, Allentown, PA) and included total glutathione peroxidase activity (GSH-Px, EC 1.11.1.9; coupled reaction at 30°C with GSH reductase using cumene hydroperoxide), selenium-dependent GSH-Px activity (using H_2O_2 as a substrate rather than cumene hydroperoxide), glutathione reductase (GSSG-Red, EC 1.6.4.2), alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), creatine phosphokinase (CK; EC 2.7.3.2), and lactate dehydrogenase-L (LDH-L; EC 1.1.1.27). Three of these enzymes are linked to hepatotoxicity in birds (ALT, AST, and LDH-L), and CK to neural and muscle tissue alterations. Other plasma constituents measured included albumin, total protein, glucose, uric acid, creatinine, cholesterol, triglycerides, calcium and inorganic phosphorus (Hoffman et al., 2000).

Portions of the liver and kidney were prepared as described for liver by Hoffman and Heinz (1998) and activities of GSH-Px (coupled reaction at 30°C with GSSG-Red using cumene hydroperoxide), Se-dependent GSH-Px (using H_2O_2 as a substrate rather than cumene hydroperoxide), and GSSG-Red were recorded spectrophotometrically by micromethods using a centrifugal analyzer as described by Jaskot et al. (1983). Activities of glutathione-*S*-transferase (GSH-Trans; EC 2.5.1.18) and glucose-6-phosphate dehydrogenase (G-6-PDH; EC 1.1.1.49), and reduced glutathione (GSH), total hepatic sulfhydryl concentrations (TSH), oxidized glutathione (GSSG), protein-bound sulfhydryl (PBSH) concentrations, and thiobarbituric acid reactive substances (TBARS) as an estimate of hepatic lipid peroxidation were determined according to Hoffman and Heinz (1998).

Serum samples were collected before ducks were euthanized and analyzed for total protein (Marshfield Laboratories, Marshfield, WI). Serum protein electrophoresis was performed on agarose films according to manufacturer's instructions (Beckman Instruments, Inc., Application Manual, Brea, CA). Films were scanned at 600 nm in a Beckman Appraise (Brea, CA) densitometer to determine relative concentrations of prealbumin, albumin, α-globulin, β-globulin, and γ-globulin and total protein was used to calculate absolute concentrations of fractions.

Necropsy and Histopathology

At necropsy, ducks were examined for gross lesions, and the liver, kidneys, spleen, and brain were weighed. The following tissues were collected in 10% neutral buffered formalin for histopathology: trachea, lung, liver, spleen, kidney, adrenal, gonad, esophagus, proventriculus, gizzard, small and large intestine, pancreas, cecum, cloaca, bursa, subcutaneous fat, thyroid, thymus, skeletal and cardiac muscle, salt gland, brain, skin (eyelid, crown, neck, thorax, interdigital web) maxilla, and digit including nail. Bony tissues were decalcified in hydrochloric acid and ethylenediamine tetraacetic acid (EDTA) (Surgipath, Richmond, IL) for 3 d. Tissues were sectioned at 5 μm and stained with hematoxylin and eosin for light microscopy. Additional stains, including Ziehl-Neelsen acid-fast, Hall's bile stain, Taylor's gram stain, Perl's Prussian blue techniques for iron, Grocott silver stain, and rhodamine (copper stain) were used depending on the results of initial histopathologic examination. Portions of liver and kidney were frozen at –80°C for biochemistries and liver, kidney, brain, and skeletal muscle were frozen at –20°C for Se analysis.

Selenium Analysis

Selenium concentrations in feed, tissue, and blood samples were determined by graphite furnace atomic absorption spectrometry (Thermo Elemental M6 Solaar with FS95 autosampler, Thermo, Franklin, MA). Analysis was done at a wavelength of 196 nm with Zeeman background correction, using a palladium nitrate/magnesium nitrate matrix modifier. Subsamples of the pelleted feed were crushed, and approximately 0.5 g of each was weighed and combined with 5 ml of trace-element-grade nitric acid (Mallinkrodt AR Select, Phillipsburg, NJ). Tissue (approximately 0.5 g ww) and blood samples (approximately 0.3 g ww) were similarly weighed and combined with 5 ml and 2 ml of nitric acid, respectively. All samples (including a procedural blank and procedural spike for each 20 samples analyzed) were prepared for analysis by pressure-controlled microwave digestion (MDS 2000, CEM, Mathews, NC) and made up to 10–250 ml with deionized reagent grade 1 (Barnstead Nanopure, Dubuque, IA) water. The lower limit of detection was 0.07 ppm ww and the average percent recovery (±SE) of Se from spiked feed and tissue samples was 104 (±2.91). Selenium concentrations are given as ppm dw in tissues and ppm ww in blood. Mean (±SE) Se concentrations (ppm, as fed) in control, 10, 20, 40, 60, and 80 ppm feed mixes were 0.84 (±0.06), 11.5 (±0.42), 20.6 (±0.44), 39.4 (±1.07), 57.7 (±1.61), and 70.2 (±0.80), respectively. Average moisture contents of the tissues were 72% for liver, 76% for kidney, 79% for brain, and 74% for muscle.

Statistics

A randomization procedure was used to compare the plasma chemistry values through time among treatments in Study 2. Because the number of sample times varied among treatment groups this was considered in 2 separate analyses. First, control and low-exposure (i.e., treatments) birds were compared across all five sample periods. Then control, low- and high-exposure birds were considered across the three sample periods in which all were sampled. In implementing the randomization procedure the sum of squares treatment (SST) across all sample times from these original data was first calculated. Then individuals were randomly assigned to treatment categories such that each category retained its original sample size, and the SST for each of these random trials was recalculated. This randomization was repeated 500 times and the proportion of the random trials in which the randomized SST exceeded the value calculated from the original data was reported. This approach is functionally similar to a Fisher's exact test, and as such is nonparametric and accounts for repeated measurements within individuals.

It was suspected that several of the plasma chemistry variables would tend to vary with body mass or condition. Further, all birds changed in body weight through the course of the trial and this relationship may have varied among treatments. Therefore, these analyses were repeated controlling for variation in body weight. To remove the variation in body weight within and among treatments, regression was used to establish the relationship between body mass and plasma chemistry values. The residuals were then calculated from these regressions and used as the dependent variables in subsequent analyses. These residual analyses were interpreted with caution. Because the biochemical response is functionally a correlational study, one cannot infer cause and effect relationships across correlated variables. Thus, if both the original analyses and the residual analyses imply a treatment effect, then one can clearly conclude a direct effect on that parameter. However, in cases where the original analysis implies an effect, and the residual analysis does not, one cannot determine if the original result was produced by the change in body mass, or was simply correlated with the change in body mass.

Rank-transformed data were used in factorial analysis of variance (ANOVA) with gender, dose, and gender × dose interaction terms to test for differences in the following variables in Study 2: organ weights, immune function responses, serum proteins, liver and kidney biochemistries, and Se concentrations in liver, kidney, brain, and muscle. A similar analysis was used to conduct post hoc comparisons of plasma biochemistries at the last sampling time and Se concentrations in tissues in the low and high-exposure groups of Study 2. Nonsignificant interaction terms were not included in the final models. Differences were considered statistically significant at $p < .05$.

RESULTS

Body and Organ Weights, Gross Lesions

Study 1 ducks and the high-exposure group in Study 2 quickly lost weight (Figure 1). One duck in Study 1 was euthanized after

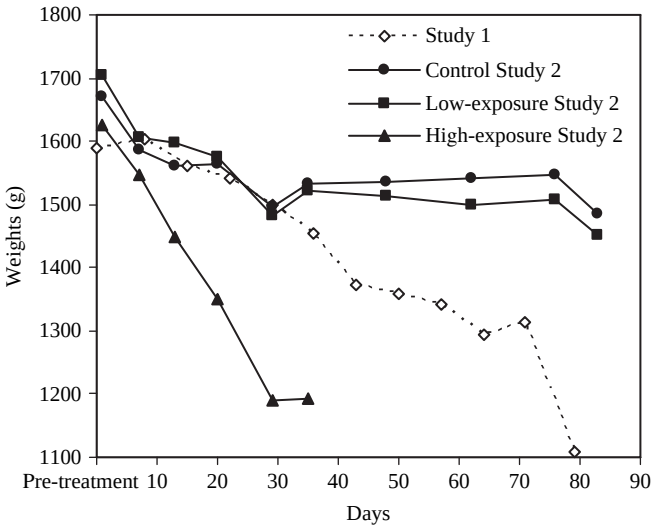

FIG. 1. Common eider weights, Studies 1 and 2. Study 1 (n = 3), control Study 2 (n = 5), low-exposure Study 2 (n = 7), high-exposure Study 2 (n = 6 through d 29, n = 4 on d 35).

60 d, having lost 27% of its body weight. The other 2 ducks were euthanized on d 78 after losing 29 and 30% of their body weight. One duck in the high-exposure group of Study 2 died on d 30, and 1 was euthanized on d 32 because it developed a staggering gait and had lost 35% of its body weight. Average weight loss in the remainder of the high-exposure group was 28% when they were euthanized on d 35. Gross lesions included diminished or absent subcutaneous and abdominal fat reserves in all Study 1 ducks and high-exposure ducks in Study 2, and absence of thymus in 3/6 high-exposure ducks in Study 2. Missing digital nails were noted in 2/3 of the ducks in Study 1 and 1/6 in the high-exposure group in Study 2. One duck in Study 1 had alopecia on the ventral surface of the neck. Spleen weights differed by exposure group, but not by gender. Spleens of ducks in the high-exposure group of Study 2 weighed significantly less than those of the low-exposure group (0.75 versus 1.57 g), but splenic weights of controls (1.12 g) did not differ from either of the Se-exposed groups. Weights of liver, kidney, and brain differed by gender (M > F), but not among exposure groups.

Microscopic Lesions

The principal histopathologic lesions in ducks on Se diets were hepatopathy, atrophy of lymphoid organs (including spleen, bursa of Fabricius, and thymus), and heterophilic necrosis of feather pulp and feather epithelium (Table 1). Hepatic lesions in 5/6 high-exposure birds in Study 2 included diffuse, moderate to marked hepatocellular vacuolar change (micro- and macrovesicular lipid accumulation and hydropic change), resulting in diffuse hepatocellular swelling with compression of sinusoids; marked Kupffer cell hypertrophy and hyperplasia; and hemosiderosis of Kupffer cells and periportal macrophages with occasional erythrophagocytosis. Similar lesions were seen in all 3 ducks in Study 1 and similar but milder changes were seen in 4/7 low-exposure birds in Study 2. Control ducks displayed only mild, multifocal to diffuse, hepatocellular lipid accumulation. Four of six high-exposure ducks in Study 2 had occasional single-cell necrosis of hepatocytes, a change not seen in low-exposure birds. One high-exposure duck had panlobular hepatocellular necrosis (Figure 2). This was the only bird that died spontaneously during the course of the study. In this bird, periportal and centrilobular hepatocytes had pyknotic nuclei and marked lipid accumulation; midzonal hepatocytes had increased eosinophilia, disrupted cell margins, and absent or pyknotic nuclei.

All ducks in Study 1 and high-exposure birds in Study 2 displayed either very few, markedly atrophied splenic germinal centers (compared to controls) or no splenic germinal centers at all. In Study 2, thymus was not identifiable grossly in 3/6 ducks in the high-exposure group, and diffuse, marked cortical atrophy was seen in the thymus of the other 3 and of follicles in the bursa of Fabricius of all 6 (Figure 3). In contrast to these findings, lymphoid organs in low-exposure birds of Study 2 were generally well developed and did not differ significantly from those of controls. Well-defined thymic cortices were present in all seven low-exposure birds. The cortex of the bursa of Fabricius was also well developed in 5/6 of low-exposure birds in which it was identified; only mild atrophy of the cortex was seen in the bursa of 1/7 of low-exposure birds. Five of seven low-exposure ducks had numerous well-developed splenic germinal centers similar to those in controls. In 1/7 low-exposure birds, splenic germinal centers were atrophied and reduced in number. In another low-exposure bird, splenic germinal centers, though numerous and well-developed, had central necrosis.

Heterophilic necrosis of growing feathers (Figure 4) was seen in all 3 birds in Study 1, and in all 6 high-exposure birds and 3/7 of low-exposure birds in Study 2. Affected feathers had focally extensive to diffuse necrosis of the feather epithelium and adjacent feather pulp distal to the epidermal collar, beginning at the level of formation of the barbs and rachis. Occasionally there was thrombosis of the axial artery. Areas of necrosis were heavily infiltrated by heterophils. Moderate heterophilic inflammation of the epidermal collar was sometimes present. An additional 3/7 of low-exposure birds displayed mild heterophilic inflammation of the pulp of some growing feathers. The remaining low-exposure bird, and all control birds, had no feather inflammation or necrosis.

Additional lesions in high dose birds in Study 2 included moderate to severe atrophy of fat (6/6), epidermal necrosis with parakeratosis of the maxillary nail (3/6), and epidermal necrosis of the dorsal claw of the digit (1/6). In Study 1, all 3 ducks showed severe atrophy of fat, 1/3 had epidermal necrosis of the maxillary nail, and 2/3 displayed epidermal necrosis of the digital claw. Mild to moderate atrophy of fat occurred in 3/7 of low-exposure birds in Study 2, while 1/5 of the control birds had mild atrophy of fat. No epidermal necrosis of beak or

TABLE 1
Histologic Lesions of Common Eiders Fed Diets Supplemented With Se as L-Selenomethionine, Study 2

Organ	Lesions	Lesion frequency		
		Control (n = 5)	Low-exposure (n = 7)	High-exposure (n = 6)
Liver	Moderate to marked, diffuse hepatocellular lipidosis	0[a]	4 (57%)	5 (83%)
	Panlobular hepatocellular necrosis	0	0	1 (17%)
	Hypertrophy and hyperplasia of Kupffer cells with hemosiderin accumulation	0	4 (57%)	6 (100%)
Feathers	Necrosis of the pulp and pulp epithelium distal to the epidermal collar	0	3 (43%)	6 (100%)
Maxillary nail	Partial to full-thickness epidermal necrosis with parakeratosis	0	0	3 (50%)
Digits of feet	Full-thickness epidermal necrosis of the dorsal claw	0	0	1 (17%)
Subcutaneous fat	Moderate to severe atrophy	0	1 (14%)	6 (100%)
Lymphoid organs		0	1 (14%)	6 (100%)
Spleen	Absent or very reduced numbers of germinal centers; atrophy of remaining germinal centers			
Thymus	Marked cortical atrophy	0	0	3/3[b](100%)
Bursa of Fabricius	Marked cortical atrophy	0	0/6[c]	6 (100%)

[a]Control ducks displayed mild hepatocellular lipidosis.
[b]Thymus was not identified at necropsy in three of the high-exposure eiders.
[c]Bursa of Fabricius was not identified at necropsy in one of the low-exposure eiders.

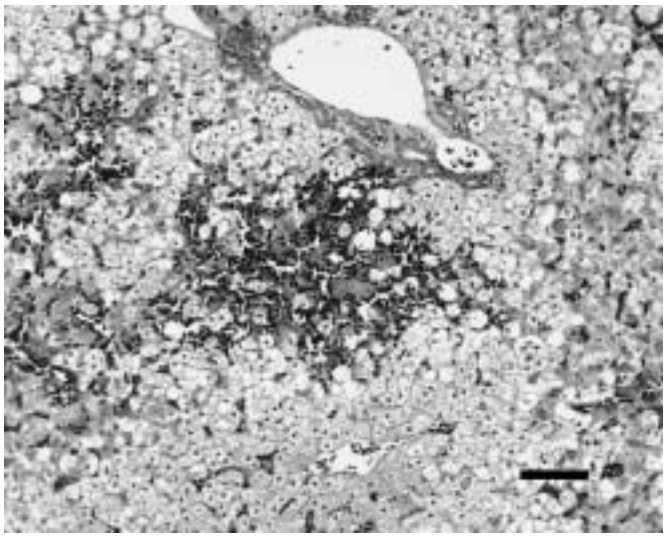

FIG. 2. Liver, high-exposure eider, Study 2. Panlobular hepatocellular necrosis. Periportal and centrolobular hepatocytes have pyknotic nuclei and marked lipid accumulation. Bar = 300 μm.

digits was seen in either the low-exposure or control birds. Lesions of uncertain significance in exposed birds were granulomas of the cecum or cecal tonsils in 5/7 of low-exposure birds, and granulomas adjacent to the cloaca and in the lung of a single high-exposure bird. Granulomas were negative for infectious organisms using Taylor's gram stain, Grocott's methenamine silver stain, and Ziehl–Neelsen acid fast stains. No intestinal granulomas were seen in control birds. In addition, fungal esophagitis due to *Candida* sp. fungi was seen in one high-exposure bird.

Incidental lesions included a single polycystic kidney in a control bird, and multifocal moderate pancreatic fibrosis in a second control bird. All birds in Study 2 showed mild lymphohistiocytic periportal hepatitis, mild biliary hyperplasia, and mild accumulations of pigmented cytoplasmic granules in hepatocytes (identified as copper with rhodamine stain). No significant changes were seen in control or exposed birds in the following tissues: proventriculus, ventriculus, duodenum, pancreas, ileum including Meckel's diverticulum, kidney, thyroid, heart, cloaca, adrenal, testes, ovaries, brain, trachea, and salt gland.

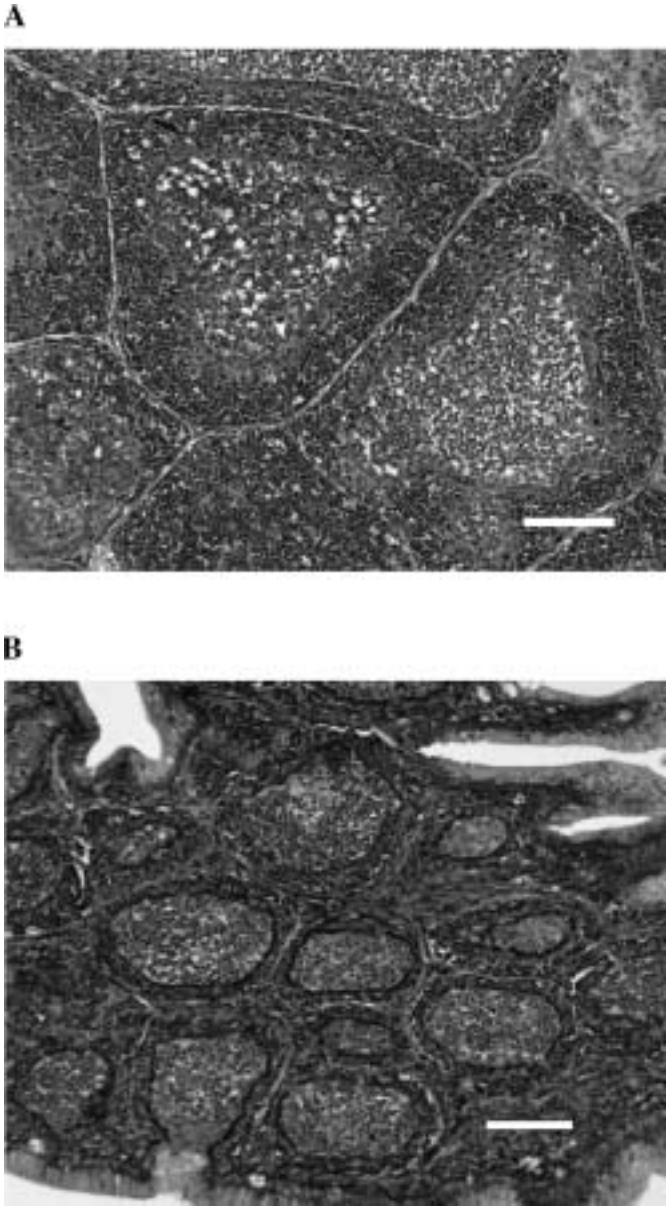

FIG. 3. (A) Bursa of Fabricius, control eider, Study 2. (B) Bursa of Fabricius, high-exposure eider, Study 2, exhibiting marked atrophy. Bars = 200 μm.

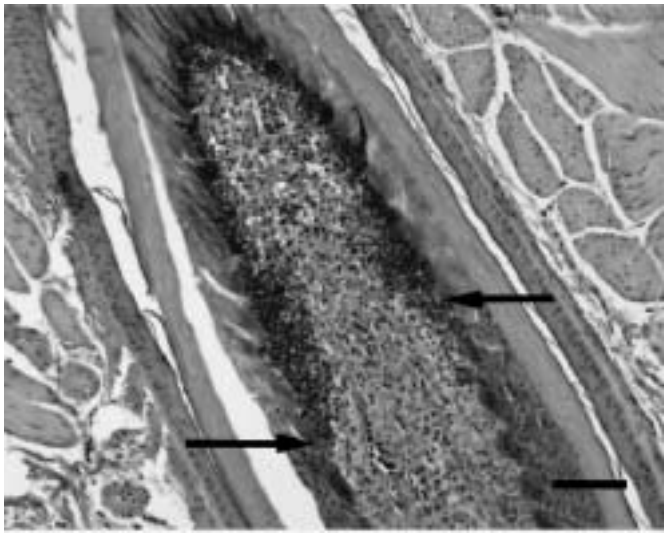

FIG. 4. Feather follicle, high-exposure eider, Study 2, exhibiting diffuse necrosis of feather epithelium beginning at the level of the formation of barbs and rachis (arrow). The necrotic epithelium is heavily infiltrated by heterophils. Bar = 150 μm.

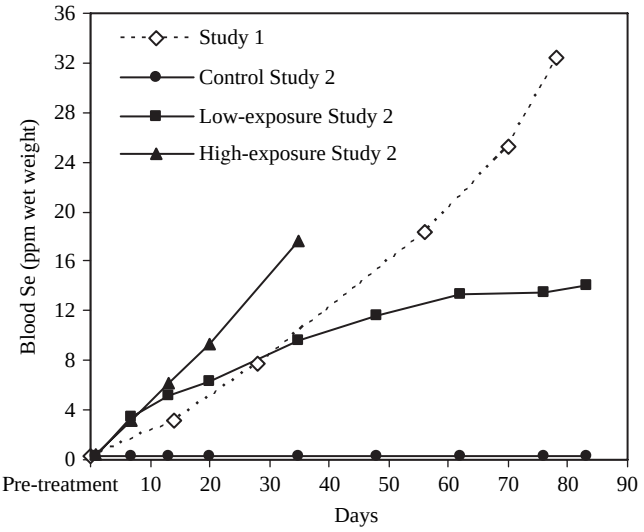

FIG. 5. Blood Se concentrations in common eiders, Studies 1 and 2. Study 1 (n = 3), control Study 2 (n = 5), low-exposure Study 2 (n = 7), high-exposure Study 2 (n = 6 through d 20, n = 4 on d 35; 1 high-exposure duck euthanized on d 32 had a blood Se concentration of 16.6 ppm).

Blood Se Concentrations, Plasma Biochemistries, Hematocrit, and Serum Electrophoresis

Mean Se concentrations in blood were <0.4 ppm in preexposure samples from all ducks and in controls, and reached 32.5 ppm in Study 1 and 14 ppm and 17.5 ppm in the low- and high-exposure groups, respectively, in Study 2 (Figure 5). When controlled for body weight during the course of Study 2, total GSH-peroxidase activity, Se-dependent GSH-peroxidase activity, and total protein concentrations in plasma differed significantly between control and low-exposure groups across five sampling times, and creatinine levels differed significantly among three treatment groups across three sampling times (Table 2). Triglycerides differed by gender in an inconsistent manner in the low- and high-exposure groups at the last sample collection. In the low-exposure group, females had greater triglycerides (54.8 vs. 48.2 mg/dl in males), while in the high-exposure group, males had greater triglycerides (71.4 vs. 29 mg/dl in females). Hematocrit exhibited treatment differences

TABLE 2
Effect of Dietary Se on Plasma Enzymes and Biochemistries (Mean and SE) In Common Eiders, Study 2

Days	Preexposure	7	35[a]	62	83
Total GSH-peroxidase (IU/L)[b]					
Control	673(122)	724(101)	876(168)	876(91.3)	880(113)
Low-exposure	750(174)	1208(188)	1221(198)	2716(466)	3265(838)
High-exposure	660(56.3)	894(133)	2788(544)		
Se-dependent GSH-peroxidase (IU/L)[b]					
Control	737(127)	972(150)	1060(200)	936(128)	885(126)
Low-exposure	783(209)	1491(216)	1557(217)	2926(495)	3427(875)
High-exposure	714(61.8)	1102(194)	3290(553)		
GSSG-reductase (IU/L)					
Control	29.8(1.74)	26.7(4.35)	23.1(2.57)	32.4(6.59)	28.3(3.73)
Low-exposure	26.8(2.50)	23.1(3.17)	18.4(3.15)	34.9(5.50)	33.1(3.52)
High-exposure	34.3(5.21)	26.6(4.58)	43.7(11.8)		
AP (IU/L)					
Control	60.2(15.6)	54.0(12.5)	50.2(12.3)	45.4(8.80)	44.0(9.17)
Low-exposure	58.8(11.5)	59.1(11.7)	41.6(3.89)	41.4(6.14)	50.1(9.81)
High-exposure	53.0(5.91)	43.8(3.44)	49.2(12.9)		
ALT (IU/L)					
Control	10.8(1.02)	11.3(1.20)	12.2(0.80)	21.8(2.46)	14.8(1.66)
Low-exposure	12.1(0.80)	10.4(1.36)	12.6(1.09)	31.7(4.17)	19.6(3.75)
High-exposure	16.2(0.91)[c]	9.50(0.67)	15.0(2.21)		
AST (IU/L)					
Control	21.8(2.63)	20.2(2.60)	16.0(1.47)	18.6(2.29)	17.4(2.56)
Low-exposure	20.4(0.92)	21.4(4.49)	17.8(4.32)	23.4(4.57)	26.4(9.56)
High-exposure	28.2(3.70)	18.0(1.50)	21.8(3.90)		
CK (IU/L)					
Control	140(16.4)	212(53.9)	279(121)	380(173)	365(144)
Low-exposure	142(14.2)	180(25.4)	174(37.8)	216(42.2)	290(106)
High-exposure	235(40.9)	179(28.2)	373(98.2)		
LDH-L (IU/L)					
Control	162(19.5)	169(26.9)	134(17.9)	173(29.0)	158(19.4)
Low-exposure	127(4.56)	145(13.9)	104(10.9)	152(17.1)	133(21.6)
High-exposure	198(39.8)	163(37.5)	258(114)		
Cholesterol (mg/dl)					
Control	297(9.46)	307(18.4)	281(8.38)	234(10.8)	286(21.9)
Low-exposure	310(16.9)	307(25.5)	317(13.6)	314(19.9)	292(20.4)
High-exposure	325(14.4)	306(28.2)	270(24.2)		
Inorganic phosphorus (mg/dl)					
Control	3.94(0.61)	4.52(1.04)	4.08(0.25)	3.14(0.54)	4.68(1.26)
Low-exposure	4.43(0.45)	4.67(0.71)	4.16(0.48)	4.10(0.34)	5.08(0.77)
High-exposure	5.47(0.54)	3.43(0.37)	5.08(0.39)		
Albumin (g/dl)					
Control	1.98(0.07)	2.04(0.05)	1.94(0.02)	1.90(0.04)	1.94(0.04)
Low-exposure	2.04(0.09)	2.03(0.12)	2.00(0.05)	2.16(0.08)	2.18(0.07)
High-exposure	2.07(0.04)	2.03(0.05)	1.30(0.17)		
Total protein (g/dl)[b]					
Control	5.34(0.42)	5.50(0.21)	5.36(0.17)	5.22(0.31)	5.22(0.17)
Low-exposure	5.11(0.19)	4.93(0.42)	5.14(0.15)	6.66(0.34)	6.56(0.27)
High-exposure	5.33(0.20)	5.12(0.23)	3.52(0.47)		

(Continued)

TABLE 2
(Continued)

Days	Preexposure	7	35[a]	62	83
Calcium (mg/dl)					
Control	10.5(0.10)	11.2(0.86)	10.4(0.16)	10.5(0.17)	10.5(0.17)
Low-exposure	10.9(0.34)	11.3(0.63)	10.6(0.13)	11.6(0.25)	11.3(0.18)
High-exposure	11.0(0.27)	11.0(0.27)	9.00(0.49)		
Blood urea nitrogen (mg/dl)					
Control	0.92(0.31)	0.58(0.26)	0.54(0.19)	1.04(0.18)	0.74(0.22)
Low-exposure	0.67(0.19)	1.04(0.28)	0.78(0.22)	1.21(0.21)	1.21(0.28)
High-exposure	0.97(0.35)	0.52(0.20)	1.16(0.12)		
Uric acid (mg/dl)					
Control	6.74(1.28)	6.78(0.28)	5.40(0.35)	7.44(1.14)	7.84(1.63)
Low-exposure	6.83(1.38)	8.64(1.52)	6.07(0.88)	9.53(0.65)	8.33(1.58)
High-exposure	7.30(0.89)	5.92(1.00)	8.02(1.76)		
Triglycerides (mg/dl)					
Control	50.8(3.31)	67.2(7.26)	54.6(4.19)	48.3(1.83)	51.5(10.2)
Low-exposure	53.7(6.10)	76.5(10.5)	51.4(8.34)	48.1(6.58)	51.1(3.85)
High-exposure	48.2(7.07)	47.0 (5.16)	55.3(11.2)		
Glucose (mg/dl)					
Control	287(14.3)	249(12.6)	250(9.36)	249(8.29)	220(21.6)
Low-exposure	281(8.72)	265(11.8)	259(5.54)	260(8.68)	251(11.8)
High-exposure	304(17.7)	270(10.6)	241(9.42)		
Creatinine (mg/dl)[c]					
Control	3.94(0.65)	3.90(0.41)	4.36(0.26)	3.88(0.27)	4.26(0.39)
Low-exposure	5.24(0.21)	6.21(0.90)	4.91(0.58)	4.88(0.45)	4.84(0.68)
High-exposure	5.25(0.48)	3.87(0.35)	5.18(0.66)		

Note. Values are means (SE); control (n = 5), low-exposure (n = 7), high-exposure (n = 6 on d 0 and 7, n = 5 on d 35).
[a]Includes sample from 1 duck that was collected on d 32 before it was euthanized.
[b]Significant ($p < .05$) difference between controls and low-exposure group across five sampling times.
[c]Significantly ($p < .05$) different than controls and low-exposure group.
[d]Significant ($p < .05$) difference among the three treatment groups across first three sampling times.

across both three and five sampling times in the original randomization analysis, but not when controlled for variation in body weight. Plasma albumin differed among the three sampling times in the first analysis, but not when controlled for body weight. Thus, the differences in hematocrit and albumin among groups may have been a treatment effect or the result of a correlation with body weight. Serum total protein and electrophoresis determinations revealed that prealbumin and the albumin/globulin ratio in control and low-exposure groups were greater than in high (Table 3). Prealbumin differed by gender (F > M in low- and high-exposure groups).

Immunocompetence

Ducks in the high-exposure group exhibited significantly less swelling (0.19 mm) in response to injection of PHA in the wing web than controls (0.38 mm) or ducks in the low-exposure group (0.50 mm). Six days after inoculation, antibody titers to SRBC were not significantly different among groups. However, at the end of the study (54 d after SRBC inoculation), mean SRBC titer in the low-exposure group was significantly higher than in controls (39 versus 14). No gender differences occurred in immune function measurements.

Tissue Se and Biochemistries

Mean Se concentrations in liver were 1252 ppm dw in Study 1 and 351 and 735 ppm dw in the low and high Se exposure groups, respectively, in Study 2 (Table 4). In Study 2, Se concentrations in liver, kidney, and brain were significantly different among control, low-exposure, and high-exposure groups (Table 4). Selenium concentrations in muscle in the low- and high-exposure groups did not differ, but concentrations in both groups were significantly greater than controls. Male eiders fed Se in Study 2 had significantly greater Se concentrations in liver than females, and this was consistent in the low (392 vs. 297 ppm) and high (875 vs. 594 ppm) exposure groups. Significant differences among exposure groups

TABLE 3
Results of Total Serum Protein Analysis and Serum Protein Electrophoresis (g/dl) (Mean and SE) in Common Eiders Fed Diets Supplemented With Se as L-Selenomethionine, Study 2

Parameter	Control (n = 5)	Low-exposure (n = 7)	High-exposure (n = 4)
Total protein	4.14(0.39)	5.54(0.53)	3.30(1.01)
Prealbumin	0.45(0.07)A	0.45(0.09)A	0.10(0.04)B
Albumin	1.73(0.15)	1.99(0.17)	1.27(0.38)
Alpha-1	0.52(0.11)	0.88(0.14)	0.64(0.28)
Alpha-2	0.12(0.03)	0.17(0.02)	0.15(0.02)
Alpha-1 + –2	0.65(0.12)	1.05(0.16)	0.80(0.30)
Beta	0.34(0.03)	0.46(0.05)	0.30(0.08)
Gamma-1	0.62(0.14)	1.10(0.16)	0.53(0.18)
Gamma-2	0.34(0.08)	0.48(0.06)	0.30(0.05)
Gamma-1 + –2	0.96(0.13)	1.59(0.21)	0.83(0.21)
Albumin/ globulin	0.12(0.01)A	0.10(0.02)A	0.04(0.003)B

Note. Within rows, means not sharing a letter in common are significantly different ($p < .05$), one-way ANOVA on rank-transformed data and Student–Newman–Keuls multiple range test.

occurred for all liver biochemistries except TBARS levels, total GSH-peroxidase activity, and GGT activity, while only Se-dependent GSH peroxidase activity was affected by Se exposure in kidney (Table 5). Tissue biochemistries did not differ by gender.

DISCUSSION

Many of the controlled studies on Se exposure were done with mallards as a model for waterfowl and other water birds. A variety of experimental designs were used, differing with regard to parameters such as the form of Se used, the concentrations in feed, the time of year, and the length of time on treatment. In comparing our findings with some of the most comparable mallard studies, it is evident that common eiders in Study 1 and in the high-exposure group of Study 2 lost weight over a relatively similar time period and developed gross and microscopic lesions similar to those seen in mallards. All eiders in these 2 groups were emaciated, as have been mallards on similar dietary Se concentrations, and alopecia noted in 1 eider in Study 1 was previously reported in mallards fed 25 or 40 ppm Se (Albers et al., 1996; O'Toole & Raisbeck, 1997). Loss of digital nails occurred in 2/3 eiders in Study 1, which were on dietary Se for up to 78 d, and in 1/6 of eiders fed the high-exposure diet for up to 35 d in Study 2. Albers et al. (1996) found claw abnormalities in mallards fed 40 ppm Se for 112 d, but not in those fed 80 ppm Se for less than 84 d, and O'Toole and Raisbeck (1997) found lesions involving digital nails only in mallards that were alive after >100 d of Se exposure. Absence of thymus, noted in 3/6 eiders in the high-exposure group in Study 2, was reported in 84% of mallards that died of selenosis (Albers et al., 1996).

The frequency of histologic lesions seen in eiders fed the high-exposure diet in Study 2 was generally greater than in mallards fed 60 ppm Se, and similar to mallards fed 80 ppm Se (Albers et al., 1996; O'Toole & Raisbeck, 1997). Hepatopathy in eiders was similar in severity to findings reported by O'Toole and Raisbeck (1997) in mallards fed 60 ppm Se, but

TABLE 4
Selenium Concentrations (ppm Dry Weight) in Tissues of Common Eiders Fed Diets Supplemented With Se as L-Selenomethionine

	Liver	Kidney	Brain	Muscle
Study 1 (n = 3)				
Mean (SE)	1252 (206)	229 (15.0)	129 (20.9)	144 (25.5)
Minimum–maximum	852–1538	202–254	87.6–151	93.0–171
Study 2				
Controls (n = 5)				
Mean (SE)	5.67 (0.09)A	5.79 (0.18)A	1.38 (0.12)A	0.80 (0.20)A
Minimum–maximum	5.36–5.90	5.43–6.43	1.06–1.66	0.07–1.25
Low-exposure (n = 7)				
Mean (SE)	351 (25.1)B	81.8 (2.98)B	57.6 (1.74)B	85.4 (2.84)B
Minimum–maximum	234–408	71.4–94.0	49.8–61.9	77.1–96.0
High-exposure (n = 6)				
Mean (SE)	735 (84.6)C	235 (42.8)C	99.2 (7.62)C	88.0 (9.75)B
Minimum–maximum	374–910	143–425	79.3–126	68.9–134

Note. Within columns for Study 2, means not sharing a letter in common are significantly different ($p < .05$), one-way ANOVA on rank-transformed data and Student–Newman–Keuls multiple range test.

TABLE 5
Effect of Dietary Se on Measurements of Oxidative Stress in Liver and Kidney of Common Eiders, Study 2

	Liver			Kidney		
Dietary Se	Control (n = 5)	Low-exposure (n = 7)	High-exposure (n = 6)	Control (n = 5)	Low-exposure (n = 7)	High-exposure (n = 6)
Se (ppm dry wt)	5.67(0.09)A	351(25.1)B	735(84.6)C	5.79(0.18)A	81.8(2.98)B	235(42.80)C
TSH (μmol/g)	18.0(0.66)A	16.5(0.72)A	11.2(1.06)B	17.1(0.58)	18.0(0.43)	15.6(0.78)
GSH (μmol/g)	3.75(0.37)A	1.93(0.29)B	1.20(0.16)B	2.86(0.08)	2.89(0.10)	2.24(0.41)
PBSH (μmol/g)	14.3(0.33)A	14.5(0.85)A	9.99(0.93)B	14.3(0.57)	15.1(0.43)	13.4(0.55)
TBARS (nmol/g)	19.9(2.66)	20.8(1.34)	20.2(0.97)	13.6(0.38)	15.8(0.88)	17.2(1.69)
GSSG (μmol/g)	0.56(0.06)A	0.67(0.08)A	1.65(0.32)B	0.06(0.004)	0.06(0.004)	0.05(0.009)
GSSG/GSH	0.15(0.007)A	0.37(0.04)B	1.77(0.68)C	0.02(0.001)	0.02(0.001)	0.04(0.018)
Total GSH-peroxidase[a]	292(22.6)	398(30.6)	345(13.4)	644(29.7)	744(42.8)	820(54.3)
Se-dependent GSH-peroxidase[a]	98.6(32.0)A	183(25.6)A	294(29.2)B	554(26.0)A	656(43.5)B	719(39.4)B
GSSG-reductase[b]	26.8(1.10)A	32.8(1.02)B	38.6(5.57)B	78.2(5.89)	91.3(5.98)	102(15.0)
GSH *S*-transferase[b]	240(9.94)A	246(10.8)A	188(16.0)B	296(18.7)	273(13.2)	268(15.8)
GGT[b]	2.95(0.34)	2.40(0.22)	2.76(0.43)	46.9(3.36)	38.4(2.27)	46.8(5.16)
G-6-PDH[b]	119(16.8)A	106(12.0)A	30.7(9.21)B	12.0(1.02)	10.1(0.62)	11.0(1.13)

Note. Within rows for each tissue, means not sharing a letter in common are significantly different ($p < .05$), factorial ANOVA on rank-transformed data and Student–Newman–Keuls multiple range test.

[a]nmol NADPH oxidized/min/mg of 10,000 × g supernatant protein.

[b]nmol/min/mg of 10,000 × g supernatant protein.

the types and frequency of liver lesions varied somewhat between the 2 studies. For example, those authors found hemosiderosis of Kupffer cells in 50% of mallards in their 60 ppm Se group, whereas data in our study showed this lesion in 100% of the common eiders in the high-exposure group. In contrast to the mallard studies, biliary hyperplasia was found in eiders in both control and Se-exposed groups. Diffuse hepatocellular swelling due to hydropic change and lipidosis was noted in 83% of high-exposure eiders, whereas O'Toole and Raisbeck (1997) found diffuse hepatocellular swelling in 42% of mallards fed 60 ppm Se. Panlobular hepatocellular necrosis was seen in the only eider in the high-exposure group that died spontaneously during our study. This lesion was found in 20 of 25 mallards that died while on 80 ppm dietary Se, but in none fed 60 ppm Se, in which only single-cell necrosis of hepatocytes was found in 25% of birds (Green & Albers, 1997; O'Toole & Raisbeck, 1997).

Eiders in the high-exposure group displayed a greater severity and frequency of lesions in feather pulp and pulp epithelium than mallards (O'Toole & Raisbeck, 1997). Abnormal feathers seen in eiders in the high Se exposure group were characterized by focally extensive to diffuse necrosis of the feather epithelium and feather pulp, with concurrent intense heterophilic inflammation. Many affected feathers were detached from follicles at the skin surface, suggesting a mechanism for alopecia. This lesion was seen in 100% of high-exposure eiders and 43% of eiders in the low-exposure group, but was conspicuously absent in control birds. Splenic lymphoid depletion was more frequent in high-exposure eiders than in mallards fed 60 ppm Se, but similar in frequency to mallards fed 80 ppm Se (Green & Albers, 1997; O'Toole & Raisbeck, 1997). A striking finding in eiders in the high-exposure group was that 100% had marked atrophy and cortical lymphocyte depletion in both thymus and bursa of Fabricius. Atrophy of lymphoid aggregates in the bursa of Fabricius was seen in 33% of mallards fed 60 ppm Se (O'Toole & Raisbeck, 1997). For the most part, thymus was not evaluated in mallards; however 1 mallard that died of selenosis was found to have severe thymic atrophy (Green & Albers, 1997). These findings suggest that eiders exposed to high levels of Se may suffer from some level of immune dysfunction. The finding of intestinal or multifocal granulomas in multiple Se-exposed birds in this study, but not in control birds, may reflect such a dysfunction.

Common eiders accumulated higher concentrations of Se in their tissues, particularly in the liver, than mallards. For example, in one study in which mallards were fed 80 ppm Se, mean Se concentrations in liver were 99 ppm dw (Albers et al., 1996). In another study, mallards that were fed 60 ppm Se had 60.6 ppm ww Se in liver, and lesser concentrations in other tissues (O'Toole & Raisbeck, 1997). Eiders in Study 1 and the high-exposure group of Study 2 showed hepatic Se concentrations of 1252 ppm dw (or 343 ppm ww) and 735 ppm dw (or 190 ppm ww), respectively. Common eiders on the low-exposure 20 ppm Se diet did not lose weight compared with controls and

developed few Se-related lesions, similar to results of previous studies in mallards fed 20 and 25 ppm Se, but mean Se concentrations in livers of eiders fed 20 ppm Se were 351 ppm dw (or 101 ppm ww), compared with 49 ppm dw and 29.6 ppm ww in mallards fed 20 ppm and 25 ppm, respectively (Albers et al., 1996; O'Toole & Raisbeck, 1997). In mallards fed 32 ppm Se, the hepatic Se concentration was 29 ppm ww (Hoffman et al., 1991), less than one-third the level found in eiders fed 20 ppm Se. The liver:kidney ratios of Se in common eiders exposed to dietary Se (from 3.13:1 to 5.47:1) were considerably higher than ratios reported in controlled studies with mallards and in field studies with fresh-water birds, common eiders, and king eiders, although one study reported about twice as much Se in liver than in kidney in wild spectacled eiders (Ohlendorf et al., 1988; Albers et al., 1996; O'Toole & Raisbeck, 1997; Stout et al., 2002). The Se concentrations in liver in our study were also much higher than concentrations reported in common eiders from field studies (Hollmén et al., 1998; Franson et al., 2000a; Wayland et al., 2001, 2002; Stout et al., 2002. Our finding of higher Se concentrations in livers of males fed Se than females is consistent with Stout et al. (2002), who reported higher liver Se in male spectacled eiders than females.

In studies of Se exposure in captive mallards, concentrations of Se in the blood increased rapidly and reached a plateau after about 40 to 50 d (Heinz & Fitzgerald, 1993; O'Toole & Raisbeck, 1997). In Study 2, Se in the blood of common eiders fed 20 ppm Se increased to 9.67 ppm ww after 35 d, 11.6 ppm ww after 48 d, then remained at 13–14 ppm ww for the remainder of the experiment (Figure 5). After 83 d, the mean blood Se concentration of eiders in the 20 ppm group was 14 ppm ww, quite similar to levels reported in mallards after 12 wk on 20 ppm or about 90 d on 25 ppm dietary Se (Heinz & Fitzgerald, 1993; O'Toole & Raisbeck, 1997). The mean concentration of Se in the blood Se of high-exposure eiders in Study 2 was 17.5 ppm ww after 35 d, which compares with about 16 ppm ww in mallards fed 60 ppm Se (O'Toole & Raisbeck, 1997). Thus, although the Se concentrations in the livers of eiders were much higher than in livers of mallards, concentrations in the blood of the two species were similar. In Study 1, blood Se concentrations in eiders continued to rise as Se levels in the feed were progressively greater, reaching a maximum of 32.4 ppm ww after 78 d.

Laboratory studies with mallards revealed that excess dietary Se induces oxidative stress in different stages of the life cycle (Hoffman & Heinz, 1988; Hoffman et al., 1989, 1991; Hoffman, 2002). Manifestations of the oxidative stress include alterations in hepatic glutathione metabolism towards a more oxidized state, accompanied by lipid peroxidation. As dietary and subsequent tissue concentrations of Se are gradually increased, these changes include initial elevation in plasma and hepatic GSH peroxidase activities, followed by a dose-dependent rise in the ratio of hepatic oxidized to reduced glutathione (GSSG:GSH), and ultimately increased hepatic lipid peroxidation. In the present study, similar evidence of hepatic oxidative stress occurred in common eiders and included elevated GSSG levels and GSSG:GSH ratio. However, hepatic lipid peroxidation as increased TBARS levels was not apparent in the eiders. In mallard studies, two factors accounted for the dose-dependent increase in ratio of GSSG to GSH: (1) a dose-dependent rise in oxidized glutathione by as much as 240% and (2) a dose-dependent depletion of reduced glutathione (GSH), sometimes falling to less than 25% of corresponding control values. In eiders, both of these factors played a role. Hepatic GSSG increased about threefold in the high-dose group relative to controls and GSH decreased to 32% of the corresponding control value. In mallards, a depletion of total hepatic thiols often appeared to parallel the depletion of GSH but was as much as twice that of the GSH depletion in terms of μmoles of GSH equivalents. This was also apparent in eiders, where the depletion of total hepatic thiols was three times that of GSH depletion.

Elevated plasma and hepatic glutathione peroxidase activities were apparent in eiders in the present study, as reported in mallards (Hoffman & Heinz, 1988; Hoffman et al., 1989, 1991; Hoffman, 2002). Elevated plasma GSH-peroxidase activity is associated with Se exposure and the onset of oxidative stress in birds (Hoffman et al., 1989, 1991) and mammals (Combs & Combs, 1986; Whanger, 1986; Levander, 1986). Other hepatic enzymes related to glutathione metabolism that were affected in eiders included increased GSSG reductase activity, whereas GSH *S*-transferase and G-6-PDH activities decreased in the high-dose group.

One or more of the above oxidative effects in mallards were associated with embryotoxicity and teratogenesis (4.6 ppm ww Se in eggs), reduced growth in ducklings (15 ppm ww Se in liver), diminished immune function in adults (5 ppm ww Se in liver,) and histopathological lesions (29 ppm ww Se in liver), as summarized in Hoffman (2002). Manifestations of Se-related effects on glutathione metabolism were also documented in field studies in seven species of aquatic birds and accompanying biological effects ranged from reduced growth and immune function to emaciation, histopathological lesions, and mortality (Hoffman, 2002). When evaluating different aspects of Se toxicity and oxidative stress, interactive nutritional factors appear to play an important role, such as the presence of other trace elements and dietary protein, and should, therefore, also be taken into consideration.

Both high Se exposure and Se deficiency are known to produce impairment of the immune system, while low levels of Se supplementation tend to enhance immune function (Kiremidjian-Schumacher & Stotzky, 1987). Selenium-related immune impairment has been infrequently reported in waterfowl, although experimental mallards exposed to Se in drinking water exhibited reduced delayed-type hypersensitive response to tuberculin and decreased resistance to duck hepatitis virus (Whitely & Yuill, 1989; Fairbrother & Fowles, 1990). Our finding of a decreased response to the PHA skin test in common eiders in the high-exposure group of Study 2 provides further evidence of immune function impairment associated with high

Se exposure. Although Wayland et al. (2002) found the response to the PHA skin test in common eiders to be positively related to liver Se concentrations, the range of liver Se concentrations in their study (approximately 8 to 31 ppm dw) was much lower than the Se concentrations found in experimental eiders. Data suggest enhanced immune function in eiders in the low Se exposure group because, although there was no significant difference among the treatment groups in the 6-d response to SRBC, ducks in the 20 ppm Se group had a higher titer than controls 54 d after SRBC inoculation. Enhanced immune function and prolonged maintenance of antibody titers were reported in experimental animals on dietary Se supplementation, but at much lower levels than used here in eiders (Kiremidjian-Schumacher & Stotzky, 1987; Larsen et al., 1997; Hegazy & Adachi, 2000). Further evidence of a beneficial effect of 20 ppm dietary Se in common eiders were higher concentrations of plasma total protein in this group compared with controls.

CONCLUSIONS

Although common eiders in the high-exposure group of Study 2 exhibited responses similar to those reported in studies with mallards, they accumulated much higher concentrations of Se in liver tissue. Eiders in the low Se exposure group in Study 2 did not lose weight compared to controls, developed few lesions of Se exposure, and exhibited responses (higher plasma total protein and higher SRBC titer after 54 d than controls) suggestive of a beneficial effect. This occurred despite the fact that they accumulated tissue Se concentrations previously associated with toxicity in fresh-water birds. Male eiders fed Se accumulated higher concentrations in liver than females. Common eiders and probably other sea ducks apparently possess a higher threshold, or adverse effect level, of Se in tissues than do fresh-water species. Further research is needed to examine what is behind the fundamental difference(s) between marine and fresh-water birds with regard to Se concentrations in tissues and toxicity.

REFERENCES

Albers, P. H., Green, D. E., and Sanderson, C. J. 1996. Diagnostic criteria for selenium toxicosis in aquatic birds: Dietary exposure, tissue concentrations, and macroscopic effects. *J. Wildl. Dis*. 32:468–485.

Combs, G. F., Jr., and Combs, S. B. 1986. *The role of selenium in nutrition*. Orlando, FL: Academic Press.

Eisler, R. 2000. *Handbook of chemical risk assessment: Health hazards to humans, plants, and animals.* Boca Raton, FL: Lewis.

Fairbrother, A., and Fowles, J. 1990. Subchronic effects of sodium selenite and selenomethionine on several immune functions in mallards. *Arch. Environ. Contam. Toxicol*. 19:836–844.

Fairbrother, A., Smits, J., and Grasman, K. A. 2004. Avian immunotoxicology. *J. Toxicol. Environ. Health B* 7:105–137.

Franson, J. C., Schmutz, J. A., Creekmore, L. H., and Fowler, A. C. 1999. Concentrations of selenium, mercury, and lead in blood of emperor geese in western Alaska. *Environ. Toxicol. Chem.* 18:965–969.

Franson, J. C., Hollmén, T., Poppenga, R. H., Hario, M., and Kilpi, M. 2000a. Metals and trace elements in tissues of common eiders (*Somateria mollissima*) from the Finnish archipelago. *Ornis Fennica* 77:57–63.

Franson, J. C., Hollmén, T., Poppenga, R. H., Hario, M., Kilpi, M., and Smith, M. R. 2000b. Selected trace elements and organochlorines: Some findings in blood and eggs of nesting common eiders (*Somateria mollissima*) from Finland. *Environ. Toxicol. Chem.* 19:1340–1347.

Franson, J. C., Hoffman, D. J., and Schmutz, J. A. 2002. Blood selenium concentrations and enzyme activities related to glutathione metabolism in wild emperor geese. *Environ. Toxicol. Chem.* 21:2179–2184.

Franson, J. C., Hollmén, T. E., Flint, P. L., Grand, J. B., and Lanctot, R. B. 2004. Contaminants in molting long-tailed ducks and nesting common eiders in the Beaufort Sea. *Mar. Pollut. Bull.* 48:504–513.

Grand, J. B., Franson, J. C., Flint, P. L., and Petersen, M. R. 2002. Concentrations of trace elements in eggs and blood of spectacled and common eiders on the Yukon–Kuskokwim Delta, Alaska. *Environ. Toxicol. Chem.* 21:1673–1678.

Green, D. E., and Albers, P. H. 1997. Diagnostic criteria for selenium toxicosis in aquatic birds: histologic lesions. *J. Wildl. Dis.* 33:385–404.

Hegazy, S. M., and Adachi, Y. 2000. Comparison of the effects of dietary selenium, zinc, and selenium and zinc supplementation on growth and immune response between chick groups that were inoculated with *Salmonella* and aflatoxin or *Salmonella*. *Poult. Sci*. 79:331–335.

Heinz, G. H. 1996. Selenium in birds. In *Environmental contaminants in wildlife: Interpreting tissue concentrations*, eds. W. N. Beyer, G. H. Heinz, and A. W. Redmon-Norwood, pp. 447–458. Boca Raton, FL: Lewis.

Heinz G. H., and Fitzgerald, M. A. 1993. Overwinter survival of mallards fed selenium. *Arch. Environ. Contam. Toxicol.* 25:90–94.

Henny, C. J., Rudis, D. D., Roffe, T. J., and Robinson-Wilson, E. 1995. Contaminants and sea ducks in Alaska and the circumpolar region. *Environ. Health Perspect.* 103(suppl. 4):41–49.

Hoffman, D. J. 2002. Review: Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat. Toxicol.* 57:11–26.

Hoffman, D. J., and Heinz, G. H. 1988. Embryotoxic and teratogenic effects of selenium in the diet of mallards. *J. Toxicol. Environ. Health* 24:477–490.

Hoffman, D. J., and Heinz, G. H. 1998. Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks. *Environ. Toxicol. Chem.* 17:161–166.

Hoffman, D. J., Heinz, G. H., and Krynitsky, A. J. 1989. Hepatic glutathione metabolism and lipid peroxidation in response to excess dietary selenomethionine and selenite in mallard ducklings. *J. Toxicol. Environ. Health* 27:263–271.

Hoffman, D. J., Heinz, G. H., LeCaptain, L. J., Bunck, C. M., and Green, D. E. 1991. Subchronic hepatotoxicity of selenomethionine in mallard ducks. *J. Toxicol. Environ. Health* 32:449–464.

Hoffman, D. J., Heinz, G. H., Sileo L., Audet, D. J., Campbell, J. K., LeCaptain, L. J., and Obrecht, H. H. III. 2000. Developmental toxicity of lead-contaminated sediment in Canada geese (*Branta canadensis*). *J. Toxicol. Environ. Health A* 59:235–252.

Hollmén, T., Franson, J. C., Poppenga, R. H., Hario, M., and Kilpi, M. 1998. Lead poisoning and trace elements in common eiders *Somateria mollissima* from Finland. *Wildl. Biol.* 4:193–203.

Hollmén, T. E., Franson, J. C., Flint, P. L., Grand, J. B., Lanctot, R. B., Docherty, D. E., and Wilson, H. M. 2003. An adenovirus linked to mortality and disease in long-tailed ducks (*Clangula hyemalis*) in Alaska. *Avian Dis.* 47:1434–1440.

Jaskot, R. H., Charlet, E. G., Grose, E. C., Grady, M. A., and Roycroft, J. H. 1983. An automated analysis of glutathione peroxidase, S-transferase, and reductase activity in animal tissue. *J. Anal. Toxicol.* 7:86–88.

Kiremidjian-Schumacher, L., and Stotzky, G. 1987. Review: Selenium and immune responses. *Environ. Res.* 42:277–303.

Larsen, C. T., Pierson, F. W., and Gross, W. B. 1997. Effect of dietary selenium on the response of stressed and unstressed chickens to *Escherichia coli* challenge and antigen. *Biol. Trace Element Res*. 58:169–176.

Levander, O. A. 1986. The need for measures of selenium status. *J. Am. Coll. Toxicol*. 5:37–44.

Ohlendorf, H. M. 1996. Selenium. In *Noninfectious diseases of wildlife*, eds. A. Fairbrother, L. N. Locke, and G. L. Hoff, pp. 128–140. Ames: Iowa State University Press.

Ohlendorf, H. M., Kilness, A. W., Simmons, J. L., Stroud, R. K., Hoffman, D. J., and Moore, J. F. 1988. Selenium toxicosis in wild aquatic birds. *J. Toxicol. Environ. Health* 24:67–92.

O'Toole, D., and Raisbeck, M. F. 1997. Experimentally induced selenosis of adult mallard ducks: Clinical signs, lesions, and toxicology. *Vet. Pathol.* 34:330–340.

Skorupa, J. P. 1998. Selenium poisoning of fish and wildlife in nature: Lessons from twelve real-world examples. In *Environmental chemistry of selenium*, eds. W. T. Frankenberger, Jr., and R. A. Engberg, pp. 315–354. New York: Marcel Dekker.

Smits, J. E., Bortolotti, G. R., and Tella, J. L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* 13:567–572.

Stout, J. H., Trust, K. A., Cochrane, J. F., Suydam, R. S., and Quakenbush, L. T. 2002. Environmental contaminants in four eider species from Alaska and arctic Russia. *Environ. Pollut.* 119:215–226.

Trust, K. A., Rummel, K. T., Scheuhammer, A. M., Brisbin, I. L., Jr., and Hooper, M. J. 2000. Contaminant exposure and biomarker responses in spectacled eiders (*Somateria fischeri*) from St. Lawrence Island, Alaska. *Arch. Environ. Contam. Toxicol*. 38:107–113.

U.S. Department of the Interior. 1998. *Guidelines for interpretation of the biological effects of selected constituents in biota, water, and sediment.* Denver, CO: Bureau of Reclamation, National Irrigation Water Quality Program Information Report No. 3.

Wayland, M., Garcia-Fernandez, A. J., Neugebauer, E., and Gilchrist, H. G. 2001. Concentrations of cadmium, mercury, and selenium in blood, liver and kidney of common eider ducks from the Canadian arctic. *Environ. Monit. Assess.* 71:255–267.

Wayland, M., Gilchrist, H. G., Marchant, T., Keating, J., and Smits, J. E. 2002. Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. *Environ. Res.* A 90:47–60.

Wayland, M., Smits, J. E. G., Gilchrist, G. H., Marchant, T., and Keating, J. 2003. Biomarker responses in nesting, common eiders in the Canadian arctic in relation to tissue cadmium, mercury and selenium concentrations. *Ecotoxicol.* 12:225–237.

Whanger, P. D. 1986. Some comparative aspects of selenite and selenomethionine metabolism. *J. Am. Coll. Toxicol.* 5:101–110.

Whiteley, P. L., and Yuill, T. M. 1989. *Immune function and disease resistance of waterfowl using evaporation pond systems in the southern San Joaquin Valley, California, 1986–89*. Final report, U.S. Fish and Wildlife Service Cooperative Agreement 14-16-0009-1511. Madison, WI: USGS National Wildlife Health Center.

Wilson, H. M., Petersen, M. R., and Troy, D. 2004. Concentrations of metals and trace elements in blood of spectacled and king eiders in northern Alaska, USA. *Environ. Toxicol. Chem.* 23:408–414.