An Investigation into the Ecotoxicology of Selenium Bioaccumulation in Birds

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Project 00-019

Executive Summary

This proposal will continue and expand current research to provide comparative avian data on Selenium (Se) bioaccumulation, embryo malformations, biochemical forms of sleneium responsible, and mechamism of selenium toxicity. The main focus of the work is being conducted as part of the Ph.D. thesis research of Steven Detwiler. Close collaboration with the U.S. Fish and Wildlife Service, and the labs of Drs. Teresa Fan and Rick Higashi at UC Davis, are providing continuity of these results with ecotoxicological studies of food web metabolism and bioaccumulation.

Marked variation between wild bird species exists in sensitivity to the teratogenic effects of selenium. Our working hypothesis is that more tolerant species have the ability to distinguish between methionine and selenomethionine during protein synthesis; and as a result, the embryos of resistant species incorporate less Se into proteins, and are thus protected from the teratogenic effects of Se. As one measure of evaluation, both wild bird eggs and does chicken eggs have been separated into yolk, albumin, and embryo fractions and analyzed for Se to evaluate the ability of different species to prevent Se incorporation into embryonic proteins and tissues.

Work to this point has included the examination of 231 eggs from 9 species of wild birds nesting in known, or suspected, selenium contaminated environments. The egg contents have been fractionated into embryonic, yolk, and albumin compartments and extracted for determination of total selenium and protein-associated selenium, to quantify the differences between species with respect to the ability of embryos to partition selenium into (or away from) protein fractions during development. All eggs from this research have been processed and archived for future analyses as directed by results of ongoing research, and as new methods are developed.

The wild bird data provide comparative data to evaluate the hypothesis that Se tolerant species can exclude Se from becoming incorporated into embryonic tissues. The preliminary data are consistent with this premise, as shown in Figure 2 (not included here), in which American Avocet (AMAV) embryos incorporate a smaller fraction of total Se in the egg into embryonic tissues than do two more sensitive species, Killdeer (KILL) or Black-necked Stilts (BNST). Initial data suggest that ore tolerant avian species (American avocet) effectively exclude selenium from embryonic tissues relative to more sensitive species with similar dietary ecology. Total selenium analyses for all wild egg fractions have been completed and results are being compiled and will be presented in the final project report this year.

A laboratory feeding trail has been conducted exposing chicken to SelenoMax selenized yeast, containing about 70% of selenium as selenomethionine. The dietary transfer of selenium into eggs, the chemical forms of selenium sequestered or utilized, and the net

toxic effects to embryos are being measured in check eggs to compare with wild birds. The studies with chicken eggs are evaluating the incorporation of dietary selenium into eggs and into the embryo as a function of the total and protein bound selenium present in fresh eggs. Analyses of chicken eggs have yet to be completed. However, the feeding trail successfully produced eggs from a range of dietary exposures (control plus 3 treatments), including overt terata similar to those observed in wild birds or other studies of chickens fed organic selenium. These results shall also appear in the final project-year report this June.

Hen weights during the feeding trail were monitored before and after each does administration. Dose-dependent cachexia (wasting syndrome) was observed in the study hens. Measurements of chicken embryonic masses at defined incubation periods were also collected to detect dose-dependent retardation of embryogenesis (as suggested in the literature). Statistical analyses are incomplete to date, but it is expected that this pattern will be quantitatively confirmed for visually "normal" chicken embryos. Such results suggest a possible mechanistic link (delayed hatching) to reduced hatchability observed in wild bird eggs of exposed populations. This can be compared to artificial incubation data, as well as the robust dataset from prior filed work by USFWS.

Evaluation of the transfer of Se from compartments in fresh eggs into embryos of wild birds has provided a parallel dataset to the chicken feeding trial. The Se compartmentalization and chemical form in fresh eggs is being followed as embryos grow, synthesize new protein, and incorporate Se into protein, or, in resistant birds, prevent its incorporation into protein. More study is required to render confidence to extrapolations across species, and particularly from the lab to field results. These include investigations into realistic dietary forms (e.g., how good is selenized yeast as a surrogate for natural diet?) and comparisons across taxa (e.g., how do we effectively calibrate measurements across species from chickens to wild shorebirds?).

To help bridge this gap, a feeding trial with Mallards duplicating the chicken feeding trial is proposed for Year 2000-2001. Mallards are wild ducks for which considerable field data have been collected by USFWS. However, lab data for this species is very limited – especially with regard to sensitive determination of relevant endpoints, and in a manner consistent with our current studies looking into Se form and partitioning within the egg. A comparison will be made for the dietary transfer of Se into eggs, the Se incorporation in protein, and the sensitivity of the two species (chicken and duck) to Se-induced malformations during embryogenesis. The comparative data for these two species will provide the links for interpreting the dose response, protein bound Se, and terata observed in wild ducks, along with more resistant wild species.

The data to date assume that protein associated or protein bound Se represents Se substitution into sulfur-amino acids, especially methionine. Confirmation of this is proposed for this year, with GC/MS confirmation of the form of Se in the protein fraction of a sub-sample of study eggs. Collaboration with Dr. Teresa Fan, Department of Land, Air and Water Resources, UCD, is already established, as Steven Detwiler is conducting his Se assays in her laboratory.