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Predicting Mercury in Mallard Ducklings from Mercury in Chorioallantoic Membranes

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Methylmercury has been suspected as a cause of impaired reproduction in wild birds such as the common loon (*Gavia immer*) (Barr 1986) and common tern (*Sterna hirundo*) (Fimreite 1974), but the confounding effects of other environmental stressors has made it difficult to determine how much mercury in the eggs of these wild species is harmful. To complement the results from field studies, controlled laboratory feeding studies have been conducted with ring-necked pheasants (*Phasianus colchicus*), black ducks (*Anas rubripes*), mallards (*Anas platyrhynchos*), and chickens (*Gallus gallus*), in which the concentrations of mercury in eggs required to cause a reduction in reproductive success have been estimated (Fimreite 1971; Finley and Stendell 1978; Heinz 1979; Tejning 1967). Unfortunately, controlled laboratory studies in which breeding pairs are fed methylmercury have not been carried out with other species of birds. Given the great cost and time required to conduct such controlled breeding studies, few wild birds are likely to be tested in the near future. Consequently, the thresholds derived for pheasants, chickens, and ducks have sometimes been used to predict whether eggs collected from the nests of wild birds may contain harmful concentrations of mercury.

There are occasions when even the sampling of eggs from wild bird nests is not possible. Sometimes the eggs will have already hatched and the young will have left the nest, and, with endangered species, sampling eggs or nestlings may not be permitted. Even when a sample egg can be collected from the nest of a wild bird and the mercury concentration in that egg compared to the laboratory-derived thresholds for reproductive impairment, additional information on the mercury levels in other eggs from that nest would be helpful in determining whether harmful levels of mercury were present in the clutch. For example, in field studies it would be very useful to know how much mercury was in eggs that hatched versus those that did not hatch, but obviously it is not possible to collect an egg for mercury analysis and still leave it in the nest to see if it hatches.

The measurement of mercury levels in chorioallantoic membranes offers a possible way to estimate how much mercury was in a chick that hatched from an egg, and also in the whole fresh egg itself. While an embryo is developing, wastes are collected in a sac called the chorioallantoic membranes. When a chick hatches, this sac is left

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behind with the eggshell. Although there are times when this sac may fall out of the eggshell during hatching and become lost or stuck to nest material, often it remains inside the eggshell and can be collected for contaminant analysis. In field studies with great blue herons (*Ardea herodias)*, Cobb et al. (1994, 1995) demonstrated that many of the hatched heron eggshells contained chorioallantoic membranes that could be used for contaminant analyses. Pastor et al. (1996) found that levels of some organochlorines in chorioallantoic membranes correlated with levels in the rest of the egg contents (yolk plus embryo) of Audouin's gull (*Larus audouinni*) eggs collected in the wild at the pipping stage.

Our goal was to determine whether there was any promise in using mercury concentrations in chorioallantoic membranes to predict mercury concentrations in hatchlings.

MATERIALS AND METHODS

We fed methylmercury to captive mallards in order to generate a broad range of mercury levels in eggs, allowed the eggs to hatch normally, and then compared mercury concentrations in the hatchling versus the chorioallantoic membranes left behind in the eggshell. Fourteen pairs of adult mallards, purchased from the Kidder Game Farm in Milton, WI, were placed in separate 1-m^2, outdoor breeding pens. Eight of these pairs were fed an uncontaminated diet, and one egg was collected from each pair to establish the level of mercury in control eggs. Total mercury was measured in these eight whole fresh eggs. Of the remaining six pairs, two pairs were fed either 5, 10, or 20 ppm mercury, as methylmercury chloride, mixed into a commercial game bird breeder diet that contained about 20% crude protein, 2.5% crude fat, and 7% crude fiber (Purina Mills, Inc., St. Louis, MO). The diets were prepared by dissolving the methylmercury chloride in acetone and then into a larger volume of corn oil (Hoffmann and Heinz 1998). Once on the mercury-treated diets, eggs were collected from each of these six pairs for 20 days and were hatched in an artificial incubator. We saved the newly-hatched duckling and the associated chorioallantoic membranes from the same egg from 12 mercury-treated eggs (one egg from one pair, two eggs from four pairs, and three eggs from one pair). In addition to using the three dietary treatments (5, 10, and 20 ppm mercury) to generate a range of mercury levels in the 12 analyzed eggs, some of the 12 eggs were ones that were laid early, some laid in the middle, and some laid late in the 20 days of mercury treatment. One additional egg was collected from one of the six pairs on the day prior to the start of the mercury treatments. This control egg was incubated and allowed to hatch and the chorioallantoic membranes and duckling were analyzed separately for total mercury.

Total mercury was analyzed by flow injection atomic absorption spectrophotometry at Moss Landing Marine Laboratories (Moss Landing, CA). Method blanks contained no detectable mercury. The percent recovery of mercury from a certified reference material (NRCC dogfish muscle, Dorm-2) ranged between 102 and 105%.

Recovery of mercury from spiked hatchlings and chorioallantoic membranes ranged from 96.9 to 109%. The relative percent difference between duplicate analyses of hatchlings and chorioallantoic membranes ranged from 2.63 to 5.26%. Concentrations of total mercury in membranes and ducklings are expressed on a dry-weight basis because the chorioallantoic membranes can be in various degrees of dryness when collected and might generally be partially desiccated if a nest were checked in the field. The relation between mercury in chorioallantoic membranes and hatchlings was examined by regression analysis of the logs of the mercury residues.

RESULTS AND DISCUSSION

Seven of the eight whole control eggs contained non-detectable concentrations of total mercury (all less than 0.09 ppm on a dry-weight basis). The eighth whole control egg contained only 0.22 ppm mercury. For the control egg that was allowed to hatch, neither the newly hatched duckling nor the chorioallantoic membranes contained detectable concentrations of mercury (also with a detection limit of 0.09 ppm). From the female that laid the control egg that was allowed to hatch (the egg was laid the day prior to the start of her 5 ppm mercury diet), we also analyzed the hatched duckling and chorioallantoic membranes from her sixth egg laid once on the mercury diet, and the mercury concentrations were 11.8 and 9.57 ppm, respectively. Based on these findings for control eggs, we conclude that the mercury found in the ducklings and chorioallantoic membranes from the 12 eggs laid by mercury-treated females came from the mercury diets fed to the females.

When the data from the 12 eggs laid by mercury-treated females were expressed as common logarithms, a linear equation was created by which the concentration of mercury in a duckling could be predicted from the concentration of mercury in the chorioallantoic membranes from the same egg (Figure 1).

The fact that the R^2 value for the transformed data was not higher than 0.58 suggests that ducklings from eggs with similar concentrations of mercury deposited somewhat variable amounts of mercury in their chorioallantoic membranes. Additional work with larger sample sizes might provide a better R^2 value and predictive equation. The dotted lines around the solid line in Figure 1 represent the 95% confidence limits; these limits show how variable one might expect the predicted value for mercury in the duckling to be at a given level of mercury in the chorioallantoic membranes.

If it were not possible to collect a sample egg from a clutch of wild bird eggs, it seems that the collection of the chorioallantoic membranes could be substituted, and the mercury predicted to be in the chick or whole egg could be compared to the thresholds of mercury that have been shown to cause harm in pheasants, chickens, and ducks in controlled laboratory studies. Simple mathematical calculations, based on the dry weight of the chorioallantoic membranes and the mercury concentration in the membranes, plus the weight of and estimated mercury in the chick, could be

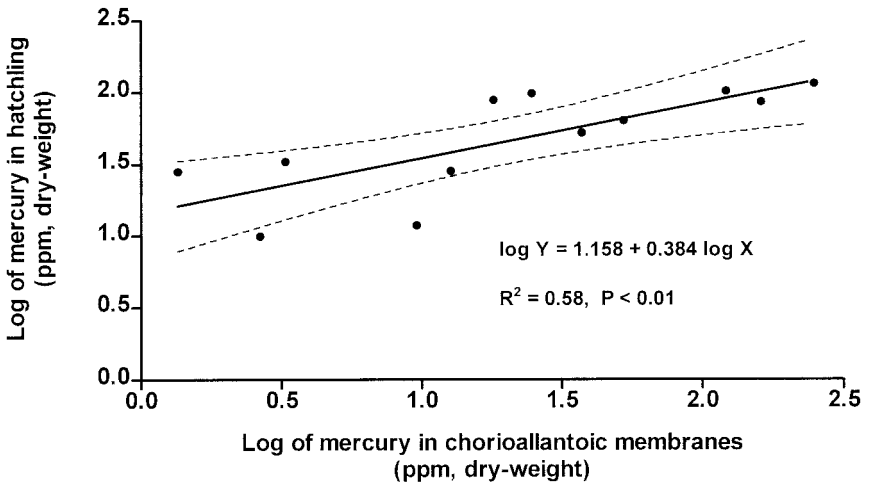

Figure 1. Predictive equation and 95% confidence limits, using log-transformed data, of mercury concentrations in ducklings based on mercury concentrations in chorioallantoic membranes of eggs laid by female mallards fed 5, 10, or 20 ppm mercury as methylmercury chloride.

used to estimate the concentration of mercury in the whole egg when the egg was laid.

Another use of chorioallantoic membranes in field studies might be to compare the concentrations of mercury in hatched eggs to levels in unhatched eggs (from the same nest or different nests). Unhatched eggs sometimes remain in the nest for some time and can be analyzed for mercury, with adjustments for moisture loss being used to correct the ppm mercury back to what it was when the egg was laid. However, the hatched eggs obviously are unavailable for analysis. In addition, precocial chicks from the hatched eggs may have left the nest, and altricial chicks still in the nest may have grown and thus altered their mercury burdens. One could use the chorioallantoic membranes left behind in hatched eggs to estimate how much mercury the chick from that egg contained; then a comparison of mercury between hatched and unhatched eggs within the same nest or between different nests could be made.

Just how well the relation between mercury in chorioallantoic membranes and mercury in hatchlings would apply to other species of birds needs to be determined, preferably both in the lab and in the field, and with larger sample sizes than were used in our study. As long as the mercury concentrations in the chorioallantoic membranes and hatchlings were both expressed on a dry-weight basis, it would not matter how desiccated the membranes of any species were when an eggshell was found in the field. What could vary in other species is the fraction of the egg's burden of mercury that the embryo ultimately deposits in its chorioallantoic membranes. With some additional laboratory work with mallards or other species

and with some field validation of the method, the use of chorioallantoic membranes could become a tool to estimate mercury levels in wild bird eggs that otherwise might not be available for chemical analyses.

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