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Integrating Toxicity Risk in Bird Eggs and Chicks: Using Chick Down Feathers To Estimate Mercury Concentrations in Eggs

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The concentration of mercury (Hg) in eggs that causes reduced hatching success is regarded as a critical end point for Hg toxicity in birds. However, incorporating effects of in ovo mercury exposure on chick health and survival could improve risk assessment. We developed equations to predict Hg in eggs using Hg in chick down feathers, and vice versa, by assessing the relationship between Hg in feathers (0.5−32.4 μg g^{-1} fw) and eggs (0.04−2.79 μg g^{-1} fww) for three waterbird species in San Francisco Bay, CA. Feather Hg sampled from embryos of pipping eggs was highly correlated with fresh whole-egg Hg ($n = 94$, $r^2 = 0.96$). Additionally, using an egg microsampling technique, albumen Hg was correlated with feather Hg sampled from chicks in the same nest ($n = 28$, $r^2 = 0.79$). Down feather Hg in recaptured chicks (≤10 days old) was correlated with down feather Hg at hatching (≤3 days old; $n = 88$, $r^2 = 0.74$). Our results demonstrate the utility of using down feathers of chicks ≤10 days of age to nonlethally predict Hg in eggs and thus provide the ability to develop exposure thresholds for eggs that incorporate in ovo Hg's effects on both egg hatchability and subsequent chick mortality.

Introduction

Methylmercury is a potent neurotoxin, and avian reproduction is among the most sensitive toxicity end points (*1*−*3*). Impaired reproduction due to mercury contamination can be manifested in several ways, including altered parental breeding behaviors (*4, 5*); reduced egg size (*6*), clutch size (*7*), and hatching success (*8, 9*); and decreased chick health (*10, 11*), growth (*12, 13*) and survival (*14*−*17*). Toxic thresholds for mercury in eggs typically have been based on egg hatchability (*7*−*9, 18*). Yet, in ovo mercury exposure can affect not only egg survival but also subsequent chick growth, behavior, and survival after hatching (*15, 19*). For example, Kenow et al. (*11, 20*) found no effects of dietary mercury exposure on chick growth but concluded that the observed reductions in asymptotic chick mass and health indices likely were due to in ovo mercury exposure. Incorporating the effects of in ovo mercury exposure on egg hatchability and subsequent chick survival into a single tissue is difficult, because it involves translating mercury concentrations from one avian life stage to another. If this were possible, then egg toxicity thresholds potentially could be refined to incorporate mercury's in ovo effects on both eggs and chicks.

After an egg hatches, chicks may still be vulnerable to the effects of residual in ovo mercury exposure, especially shortly after hatching, when maternally deposited mercury levels are still relatively high. Thereafter, barring especially high levels of mercury in their diet, chick mercury concentrations rapidly decline as chicks age and dilute their body burden of mercury through growth and depuration of mercury into growing feathers (*21*−*23*). Chick mortality associated with mercury contamination often occurs within the first week after hatching (*15*−*17*), indicating that in ovo mercury exposure can influence posthatch survival. Incorporating this early chick mortality into egg toxicity thresholds is hampered by our inability to translate mercury concentrations in chicks to what the equivalent concentrations were in eggs.

Down feathers of newly hatched chicks are potentially useful tools for estimating mercury concentrations in the eggs from which they hatched. Down feathers are grown in ovo during the embryonic phase and, in some species, can contain about 38% of the total body burden of mercury in newly hatched chicks (*24, 25*). Mercury concentrations in down feathers can be correlated with whole-body mercury burdens in chicks (*24*) and therefore may also be useful for estimating whole-egg mercury concentrations. If down feather mercury concentrations are, in fact, correlated with concentrations in the whole egg, then down feathers could be sampled nonlethally as a proxy for fresh egg mercury concentrations in studies assessing the effect of in ovo mercury exposure on subsequent chick mortality.

In this paper, we developed equations to predict mercury concentrations in fresh eggs using mercury concentrations in chick down feathers, and vice versa. We further assessed whether mercury concentrations in down feathers changed as chicks aged, possibly due to the continued production of down feathers after hatching, in order to identify the appropriate age for feather sampling. Together, these objectives provide a means of extrapolating the toxic effects of mercury in eggs to the health and survival of chicks.

Experimental Section

Whole Egg and Chick Down Feather Mercury Concentrations. We studied American avocets (*Recurvirostra americana*; hereafter avocets), black-necked stilts (*Himantopus mexicanus*; hereafter stilts), and Forster's terns (*Sterna forsteri*; hereafter terns) during the 2005−2007 nesting seasons (April−August) in San Francisco Bay, CA (37.4°N, 122.0°W). We collected eggs at several nesting sites to yield a range of mercury concentrations common in San Francisco Bay (*17, 26*). We entered colonies weekly, marked each new nest, and determined the stage of embryo development via egg floatation (*27*). For those nests that were nearing the full incubation term (about 24 days), we randomly collected one egg from nests that were at the one- to four-star pipping stage (about 21−24 days in incubation *28*−*30*). Collecting pipping eggs within 3 days of hatching ensured that we could assess mercury concentrations in both the whole-egg homogenate (which included the entire egg contents: embryo, feathers, and any remaining egg albumen and yolk) and down feathers from the fully developed embryo. We stored eggs in the refrigerator for ≤10 days before processing. We measured the length and breadth of each egg to the nearest 0.01 mm using digital calipers and measured the total egg weight to the nearest 0.01 g on a digital balance. We then carefully cut an approximately 25 mm diameter hole in the top of the egg using clean, stainless steel scissors and removed the embryo

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and any remaining contents into a 2-ounce polypropylene jar with stainless steel forceps. Total content weight was measured with a digital balance to the nearest 0.01 g. Using tweezers, we removed about 15 down feathers [0.03 ± 0.01 (SD) g] from the mantle of each chick and placed them in polypropylene cryovials. After subsampling down feathers, the embryo and remaining egg contents were stored frozen as described above.

Microsampled Egg and Chick Down Feather Mercury Concentrations. Because mercury concentrations in down feathers collected from pipping chicks, still in the egg, could be different than mercury concentrations in down feathers collected from chicks once they had hatched, we also used a nonlethal egg sampling technique, called microsampling, to verify that egg mercury concentrations were correlated with down feathers in recently hatched chicks. We describe the methodological details of this technique elsewhere (*31*). Briefly, during our routine nest monitoring procedures (described above), we selected nests where all eggs were ≤3 days in incubation and randomly selected one egg from the clutch for microsampling (*31*). We marked the egg we microsampled with a blue permanent marker and then returned the egg to its nest. The albumen microsample was transferred to a cryovial and stored on ice until it was returned to the laboratory within 5 h. Thereafter, the albumen was stored in a freezer at −20 °C until mercury analysis.

To match the albumen sample with a down feather sample from the same chick, we returned to the nest weekly to monitor the embryo's development. When the clutch hatched, we attempted to collect down feathers (mantle) from the same chick that hatched from the microsampled egg. However, this was not always possible, especially when multiple eggs hatched in the clutch before we had revisited the nest. Therefore, we categorized the down feather sample into one of three groups: feathers known to be sampled from the chick hatching from the microsampled egg, feathers known to be sampled from a sibling egg (not microsampled), and unknown feathers sampled either from the microsampled or sibling egg. We considered the sibling and unknown feather samples still to be useful, since sibling egg mercury concentrations often are highly correlated; however, we acknowledge that intraclutch variation in mercury concentrations in down feathers can be 10−39% (*25, 32*).

Mercury Concentrations in Down Feathers as Chicks Age. In order to use down feather mercury concentrations to predict egg mercury concentrations, it is important to understand if and how mercury concentrations change in down feathers as chicks age. Therefore, we banded and recaptured tern chicks at several colonies in the south (A1, A8, and A16) and north (Pond 2) regions of San Francisco Bay weekly from nest initiation (early May) until the last tern chick fledged (late August). At each visit, we hand-captured every chick at the colony, banded newly hatched chicks with stainless steel U.S. Geological Survey leg bands or recorded band numbers from recaptured chicks, and collected 10−15 down feathers from each chick for mercury analysis. We also measured the structural size (mm) of chicks at each visit in order to estimate their age using an equation we developed based on tern chicks with known hatching dates (J. T. Ackerman, unpublished data). We measured exposed culmen and short tarsus (tarsometatarus bone) lengths with digital calipers (±0.01 mm; Fowler, Newton, MA) and flattened wing length with a wing board (±1.0 mm). These methods yielded two down feather samples from the same individual, separated by 7 days. For this analysis, we used only those chicks that were first captured at 0−3 days of age and were recaptured at 7−10 days of age.

Mercury Determination. We analyzed down feathers and remaining whole-egg homogenate samples for total mercury (U.S. Geological Survey, Davis Field Station Mercury Laboratory), since more than 95% of mercury in avian eggs and feathers is methylmercury (*33*−*35*). Prior to analysis, we dried the entire egg contents at 50−60 °C for 48 h or until completely dried and reweighed the egg contents to determine moisture content. We then ground the dried egg contents to a powder in a Wiley mill, followed by further grinding in a mortar and pestle. Albumin samples were analyzed as described by Stebbins et al. (*31*) and feathers were processed and analyzed as described by Ackerman et al. (*36, 37*). Following EPA Method 7473 (*38*), we analyzed each albumen, egg, and feather sample for total mercury on a Milestone DMA-80 Direct Mercury Analyzer (Milestone Inc., Monroe, CT). Recoveries of certified reference materials [either dogfish muscle tissue (DORM-2, 4.64 μg g^{-1}, National Research Council of Canada, Ottawa, Canada), dogfish liver (DOLT-3, 3.37 μg g^{-1}, National Research Council of Canada, Ottawa, Canada), or lobster hepatopancreas (TORT-2, 0.27 μg g^{-1}, National Research Council of Canada, Ottawa, Canada)], calibration checks, and matrix spikes respectively averaged (±SE) 98.47 ± 0.78% (n = 33), 102.36 ± 1.09% (n = 49), and 103.02 ± 1.51% (n = 17). Absolute relative percent difference for all duplicates and matrix spike duplicates respectively averaged (±SE) 5.23 ± 1.54% (n = 7) and 4.86 ± 2.10% (n = 4) for down and 3.65 ± 0.66% (n = 25) and 2.44 ± 0.45% (n = 13) for eggs.

Statistical Analysis. We reconstructed mercury concentrations in the whole egg by combining mercury concentrations determined for the down feathers sampled from pipping chicks and the remaining whole-egg homogenate. To do so, we weighed (dry weight, dw) the entire sample of down feathers removed from the embryo (M_{df}) and the remaining whole-egg homogenate (M_{eh}) separately before determining their respective mercury concentrations (dw; accuracy to 0.0001 g). We then multiplied the weight of the down feathers removed from the embryo by its specific mercury concentration ($[THg]_{df}$) and added the product of the weight of the remaining whole-egg homogenate (dw) and the average mercury concentration of three subsamples of the whole-egg homogenate ($[THg]_{eh}$). This resulted in the total mercury mass in the whole egg, and we divided this quantity by the combined weight (dw) of the removed down feathers and the remaining whole-egg homogenate to yield the mercury concentration of the reconstructed whole-egg homogenate at pipping ($[THg]_{we}$dw at pipping, eq 1).

$$[THg]_{we}\text{dw at pipping} = (M_{df}[THg]_{df} + M_{eh}[THg]_{eh})/(M_{df} + M_{eh}) \quad (1)$$

Next, we converted the mercury concentration of the reconstructed whole-egg homogenate at pipping from a dry weight to a wet weight (ww) concentration using eq 2:

$$[THg]_{we}\text{ww at pipping} = ([THg]_{we}\text{dw at pipping}) \times (1 - [\%\ \text{moisture}/100]) \quad (2)$$

Since pipping eggs may have lost a substantial amount of weight from the time of laying (due to respiration and moisture loss), we then adjusted the wet weight mercury concentration of the reconstructed whole-egg homogenate at pipping ($[THg]_{we}$ ww at pipping) to a fresh egg wet weight mercury concentration (fww) by dividing the total weight (ww) of the egg content at processing (M_{ec}) by the predicted fresh egg weight (ww) at laying (M_{fe}) and multiplying that value by the wet weight mercury concentration at pipping (following ref *39*; eq 3):

$$[THg]_{we}\text{fww} = ([THg]_{we}\text{ww at pipping}) \times (M_{ec}/M_{fe}) \quad (3)$$

The fresh egg weight (ww) was estimated using egg morphometrics following Hoyt (*40*) [$M_{fe} = 0.548 \times$ egg length $\times$ (egg width)^2].

We used analysis of covariance (ANCOVA, JMP version 4.0.4) to examine whether there was an interaction between the effects of species and the reconstructed whole-egg homogenate mercury concentrations on down feather total mercury concentrations. We then used linear regression to test whether (1) mercury concentrations in down feathers were correlated with concentrations in the reconstructed fresh whole-egg homogenate, (2) mercury concentrations in albumen were correlated with concentrations in down feathers sampled from chicks found in the same nest, and (3) mercury concentrations in down feathers sampled from recaptured chicks were correlated with concentrations in down feathers sampled from chicks captured for the first time. We used *t*-tests to determine whether regression slope coefficients differed from a value of 1. Lastly, we used ANCOVA to examine whether fresh wet weight mercury concentrations differed between pipping eggs and randomly sampled eggs, and we controlled for colony site and calendar date statistically by including them as main effects in the model. All data were ln-transformed for analysis to improve normality of residuals and homogeneity of variance, and we report all egg concentrations in fresh wet weight (fww); the average (±SE) moisture content in pipping eggs was 74.85 ± 0.20% (avocet), 74.03 ± 0.44% (stilt), and 78.71 ± 0.18% (terns). Albumen was reported in wet weight (ww), and down feathers were reported in fresh weight (fw).

Results

Whole Egg and Chick Down Feather Mercury Concentrations. We collected 94 pipping eggs (stilts, $n = 16$; avocets, $n = 35$; terns, $n = 43$) at 22 ± 1.5 (SD) days of incubation. Total mercury concentrations (mean ± SD) in down feathers were 2.88 ± 2.06 μg g^{-1} fw for avocets (range 0.47−8.75 μg g^{-1} fw), 5.96 ± 2.97 μg g^{-1} fw for stilts (range 2.61−13.69 μg g^{-1} fw), and 18.32 ± 5.60 μg g^{-1} fw for terns (range 9.20−32.39 μg g^{-1} fw). We subsampled the remaining whole-egg homogenate and determined mercury concentrations in each of the three subsamples to reduce any variation that might occur due to the advanced stage of embryo development and mercury partitioning among different tissues. However, we found little variation among our three subsamples of the remaining whole-egg homogenate; the coefficient of variation among the three egg subsamples averaged 2.5% (range 0.1−9.6%). Total mercury concentrations (mean ± SD) in the reconstructed fresh whole-egg homogenate was 0.23 ± 0.15 μg g^{-1} fww for avocets (range 0.04−0.62 μg g^{-1} fww), 0.43 ± 0.21 μg g^{-1} fww for stilts (range 0.15−0.95 μg g^{-1} fww), and 1.35 ± 0.47 μg g^{-1} fww for terns (range 0.69−2.79 μg g^{-1} fww).

To assess the relationship between total mercury concentrations in down feathers and those in the reconstructed fresh whole-egg homogenate (fww), we first tested whether there were species differences in the relationships. There was no interaction between species and reconstructed fresh whole-egg homogenate mercury concentrations on down feather total mercury concentrations (ANCOVA: species × egg, $F_{2,88} = 1.44$, $p = 0.24$; species, $F_{2,88} = 5.31$, $p = 0.01$; egg, $F_{1,88} = 306.06$, $p < 0.0001$). We therefore pooled all data among species to test whether total mercury concentrations in down feathers were correlated with concentrations in eggs. Total mercury concentrations in down feathers were highly correlated with total mercury concentrations in the reconstructed fresh whole-egg homogenate (linear regression, $n = 94$, $r^2 = 0.96$, $p < 0.0001$; Figure 1). After back-transforming the linear regression equations, the equation for predicting chick down total mercury concentrations ([THg]$_{df}$; μg g^{-1} fw) from fresh whole-egg total mercury concentrations ([THg]$_{we}$; μg g^{-1} fww) was

$$[\mathrm{THg}]_{df} = e^{2.590\pm0.025}[\mathrm{THg}]_{we}^{1.000\pm0.021} \quad (4)$$

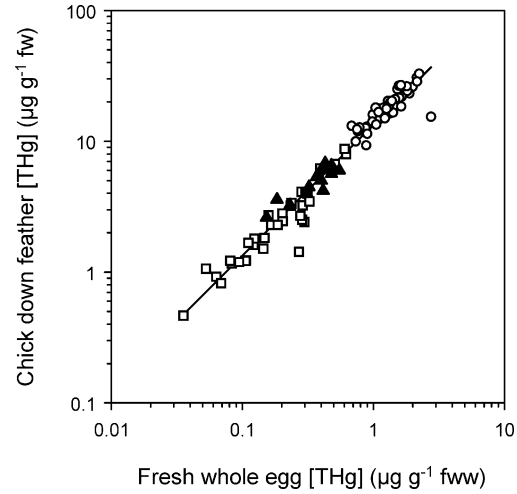

FIGURE 1. Mercury concentrations in down feathers [μg g^{-1} fresh weight (fw)] of pipping chicks were highly correlated ($r^2 = 0.96$) with mercury concentrations in the reconstructed fresh whole-egg homogenate [μg g^{-1} fresh wet weight (fww)] for Forster's terns (circles), American avocets (squares), and black-necked stilts (triangles) in South San Francisco Bay, CA. The linear regression equation describing the data was ln Y = 2.590 + 1.000(ln X).

and, conversely,

$$[\mathrm{THg}]_{we} = e^{-2.517\pm0.043}[\mathrm{THg}]_{df}^{0.962\pm0.020} \quad (5)$$

The slope coefficient (eq 4) of 1.000 ± 0.021 (SE) did not differ from 1.0 ($t_{92} = 0.01$, $p = 0.99$).

To examine the potential for differential mercury partitioning into down feathers compared to other embryo components, we also performed the linear regression analysis using the dry weight total mercury concentration of the reconstructed whole-egg homogenate at pipping, rather than the fresh wet weight egg mercury concentration. The linear regression equation (linear regression, $n = 94$, $r^2 = 0.97$, $p < 0.0001$) to predict chick down total mercury concentrations ([THg]$_{df}$; μg g^{-1} fw) from whole-egg total mercury concentrations at pipping ([THg]$_{we}$; μg g^{-1} dw at pipping) was

$$[\mathrm{THg}]_{df} = e^{0.971\pm0.025}[\mathrm{THg}]_{we}^{0.940\pm0.017} \quad (6)$$

The slope coefficient of 0.940 ± 0.017 (SE) differed from 1.0 ($t_{92} = 3.53$, $p \leq 0.001$).

Lastly, we compared fresh wet weight mercury concentrations in pipping eggs (reconstructed fresh whole-egg homogenate) to randomly sampled eggs to verify that pipping eggs were representative of the population. Mercury concentrations in pipping eggs were no different than randomly sampled eggs for avocets ($F_{1,264} = 0.46$, $p = 0.50$), stilts ($F_{1,50} = 0.09$, $p = 0.77$), or terns ($F_{1,173} = 0.01$, $p = 0.93$).

Microsampled Egg and Chick Down Feather Mercury Concentrations. We pooled data among all three species for these statistical analyses because we did not have a large enough sample to test for interactions among species (stilts, $n = 1$; avocets, $n = 5$; terns, $n = 22$). We also randomly selected a down feather sample to correlate with the albumen sample when multiple chicks had hatched from the same nest containing the microsampled egg. Albumen mercury concentrations were correlated with mercury concentrations of down feathers from chicks found in the same nest (linear regression, $n = 28$, $r^2 = 0.79$, $p < 0.0001$; Figure 2). We found similar results when we used only those samples where we were able to positively match the microsampled egg and chick as the same individual (linear regression: $n = 6$, $r^2 = 0.77$, $p = 0.02$). After back-transforming the linear regression

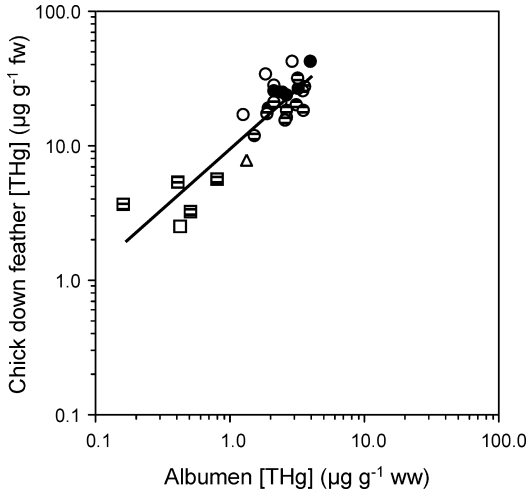

FIGURE 2. Mercury concentrations in down feathers [μg g^{-1} fresh weight (fw)] of newly hatched chicks found in the nest were correlated (r^2 = 0.79) with albumen mercury concentrations [μg g^{-1} wet weight (ww)] microsampled from an egg in the same nest when the eggs were ≤3 days incubated in South San Francisco Bay, CA. Symbol patterns [Forster's terns (circles), American avocets (squares), and black-necked stilts (triangles)] indicate whether the feathers were sampled from the same chick that hatched from the albumen microsampled egg (filled), a sibling chick from the same nest that was not microsampled during incubation (partially filled), or an unknown chick from the same nest sampled either from the microsampled or sibling egg (open). The linear regression equation describing the data was ln Y = 2.291 + 0.888(ln X).

equation from the full data set, which was based on ln-transformed data (±SE), the equation for predicting chick down feather total mercury concentrations (μg g^{-1} fw) from albumen total mercury concentrations ([THg]$_{alb}$, μg g^{-1} ww) was

$$[\mathrm{THg}]_{df} = e^{2.291\pm0.085}[\mathrm{THg}]_{alb}^{0.888\pm0.090} \quad (7)$$

and, conversely,

$$[\mathrm{THg}]_{alb} = e^{-1.919\pm0.261}[\mathrm{THg}]_{df}^{0.889\pm0.090} \quad (8)$$

The slope coefficient (eq 7) of 0.888 ± 0.090 (SE) did not differ from 1.0 (t_{26} = 1.24, p = 0.22).

Mercury Concentrations in Down Feathers as Chicks Age. To examine whether down feather mercury concentrations changed with age after hatching, we compared mercury concentrations in down feathers collected from 88 tern chicks that were first captured when they were ≤3 days of age to their down feather mercury concentrations upon their next capture 7 days later (≤10 days of age). Down feather mercury concentrations in recaptured chicks were correlated with mercury concentrations of down feathers sampled during the first capture event from recently hatched chicks (linear regression: n = 88, r^2 = 0.74, p < 0.0001; Figure 3). After back-transforming the linear regression equation, which was based on ln-transformed data (±SE), the equation for predicting recaptured chick down feather total mercury concentrations ([THg]$_{df\text{-}recapture}$; μg g^{-1} fw) from first-captured chick down feather total mercury concentrations ([THg]$_{df\text{-}first\ capture}$; μg g^{-1} fw) was

$$[\mathrm{THg}]_{df\text{-}recapture} = e^{0.384\pm0.166}[\mathrm{THg}]_{df\text{-}first\ capture}^{0.798\pm0.052} \quad (9)$$

and, conversely,

$$[\mathrm{THg}]_{df\text{-}first\ capture} = e^{0.485\pm0.176}[\mathrm{THg}]_{df\text{-}recapture}^{0.922\pm0.060} \quad (10)$$

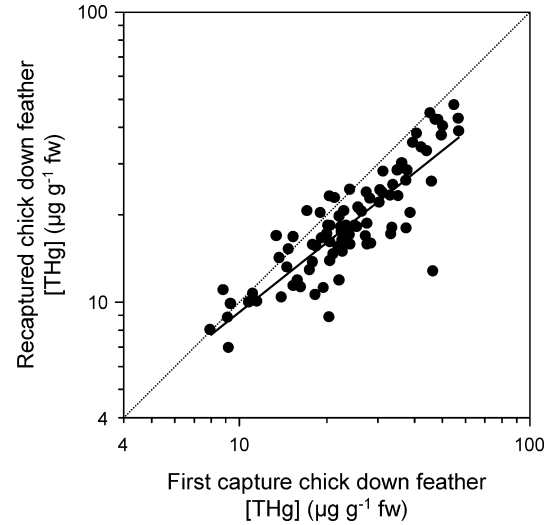

FIGURE 3. Mercury concentrations in down feathers [μg g^{-1} fresh weight (fw)] of recaptured Forster's tern chicks (≤10 days of age) were correlated (r^2 = 0.74) with mercury concentrations in down feathers of the same chicks sampled just after they hatched (≤3 days of age) in South San Francisco Bay, CA. The stippled line indicates a one-to-one line. The linear regression equation describing the data was ln Y = 0.384 + 0.798(ln X).

The slope coefficient (eq 9) of 0.798 ± 0.052 (SE) differed from 1.0 (t_{86} = 3.88, $p \leq 0.0001$).

Discussion

Using three species of waterbirds that represent different foraging guilds and trophic levels, we were able to assess the relationship between mercury concentrations in chick down feathers and eggs over a wide range of concentrations from 0.5 to 32.4 μg g^{-1} fw in feathers and 0.04 to 2.79 μg g^{-1} fww in eggs. We found a strong correlation (r^2 = 0.96) between mercury concentrations in down feathers and mercury concentrations in the reconstructed fresh whole-egg homogenate (Figure 1). Down feather mercury concentrations were elevated relative to the corresponding whole-egg homogenate mercury concentrations (dry weight) at pipping, indicating that mercury is partitioned preferentially into growing down feathers during embryo development. However, the slope of the regression was less than 1.0, indicating that at higher egg mercury concentrations (dry weight) relatively less mercury is partitioned into growing down feathers.

If down feathers are continuously produced in young chicks, then mercury concentrations in down sampled from pipping chicks that are still inside the egg could differ from chicks sampled after hatching. We therefore assessed the potential for this difference in down feathers by using a newly developed egg microsampling technique (*31*) to extract albumen during early egg development (≤3 days incubated) and compared albumen mercury concentrations to down feather mercury concentrations from newly hatched chicks in the same nest. Elsewhere we showed that mercury concentrations in albumen are highly correlated with concentrations in the whole-egg (*31*). Using this microsampling technique, we found a strong correlation (n = 28, r^2 = 0.79) between mercury concentrations in albumen and mercury concentrations in down feathers sampled from chicks in the same nest (Figure 2). For this analysis, we included down feather samples from chicks hatched from sibling eggs, as well as from chicks whose origins (microsampled egg or sibling egg) were unclear. The inclusion of these samples undoubtedly adds variance to our analysis, since intraclutch variation in mercury concentrations among eggs and chicks'

down feathers can be 10−39% (*25, 32*). However, we found similar results when we considered a smaller sample size that included only the down feather samples from chicks that were known to have hatched from the microsampled eggs ($n = 6$, $r^2 = 0.77$; Figure 2). The slope of the relationship between mercury concentrations in chick down feathers and albumen (eggs) was close to 1.0, indicating the utility of using one tissue matrix as an index of the other.

Another potential problem with using chick down feathers to predict mercury concentrations in eggs is that down feather mercury concentrations after hatching might change as chicks age. This could occur if chicks continued to produce down feathers as they grew and mercury concentrations varied with chick age. There is substantial support demonstrating that blood mercury concentrations decline rapidly as chicks age and dilute their body burden of mercury through growth and depuration of mercury into growing feathers (*12, 20−22*). However, there is less known about the timing of down feather growth and its effect on down mercury concentrations. Initially, down feathers are developed in ovo during the embryonic phase, but young chicks may also continue to produce down feathers after hatching. We therefore verified that mercury concentrations in down feathers sampled when chicks were ≤3 days of age were correlated ($r^2 = 0.74$) with concentrations in down feather samples taken from the same individual recaptured 7 days later (Figure 3). In contrast to the albumen and chick down feather relationship, the slope of the regression (0.80) between mercury concentrations in down feathers of recaptured and newly hatched chicks was less than 1.0. This suggests that down feathers sampled at 0−3 days of age might have been different than down feathers sampled at 7−10 days of age. If this were true, then chicks exposed to high in ovo mercury concentrations likely were exposed to lower mercury concentrations in their diet after hatching. This is illustrated by the fact that mercury concentrations in down feathers of recaptured chicks were nearly the same as newly hatched chicks at low mercury concentrations, but that at higher mercury concentrations recaptured chicks had relatively lower levels of mercury than newly hatched chicks. Similarly, Becker et al. (*25*) found that mercury concentrations in down feathers of highly contaminated chicks were much higher than concentrations in subsequently grown feathers, whereas there was no difference in mercury concentrations among feather types in the less contaminated chicks. Both of these results indicate that the difference in mercury concentrations between sequentially grown feathers will be larger in the more highly contaminated chicks. Nonetheless, the strong correlation we found between mercury concentrations in recaptured chicks and newly hatched chicks indicates that down feathers were still a reliable index of egg mercury concentrations, at least for chicks up to the age of 10 days posthatch.

Together, our results demonstrate the utility of using chick down feather mercury concentrations to predict concentrations in eggs, and vice versa. Whereas we have demonstrated the usefulness of this technique for three waterbird species in two families (Recurvirostridae and Laridae), future research should verify its use in other species. Our results have several applications, including for mercury monitoring programs as well as in research for assessing toxicity thresholds. Often mercury monitoring programs are based on sampling eggs (*6, 41*); however, sampling wild bird eggs is sometimes not possible due to permitting restrictions, especially with endangered species (*42*), or not desired since it necessarily results in the destruction of eggs. Currently, there are only a very few ways to sample mercury concentrations in eggs nonlethally. These techniques include using chorioallantoic membranes left behind in the eggshell posthatch (*43*) and microsampling a viable egg by extracting a small amount of albumen (*31*). However, chorioallantoic membranes can be difficult to find and should be collected shortly after hatching [(*44*); G. H. Heinz, personal communication] and albumen microsampling requires considerable training and must be done within a short time window when eggs have been incubated for ≤3 days (*31*). In contrast, sampling down feathers of chicks can occur over a longer time period (up to 10 days posthatch) and is relatively easy.

In addition to the value of this technique for monitoring mercury nonlethally, the ability to predict mercury concentrations in eggs from chick down feathers, or vice versa, can improve our assessment of egg toxicity thresholds. Because avian embryos are especially sensitive to methylmercury (reviews in refs *1−3*), egg toxicity thresholds traditionally have been developed by examining effects of in ovo mercury concentrations on egg survival (*7−9, 18*). However, chick growth (*12, 13*), behavior (*45*), and survival (*5, 7, 16, 46*) also can be affected by methylmercury. The ability to combine both of these sensitive reproductive end points into a single tissue matrix could improve our understanding of mercury toxicity levels that cause reproductive impairment. For instance, in ovo mercury concentrations that impair egg hatchability are likely to be higher than those concentrations that could impair subsequent chick growth and survival, since the chick must first hatch before it has the opportunity for its growth and survival to be affected by residual mercury exposure.

To illustrate the utility of this tissue conversion equation, we used the mercury concentration in eggs commonly associated with impaired hatchability to estimate the corresponding mercury concentration that would occur in down feathers of a newly hatched chick. Egg mercury concentrations >1.0 $\mu g\ g^{-1}$ ww often cause impaired hatchability and embryonic mortality in birds (review in ref *3*). Using our equation developed in this paper (eq 4), a concentration of 1.0 $\mu g\ g^{-1}$ ww in the whole fresh egg is equivalent to 13.3 (13.0−13.7) $\mu g\ g^{-1}$ fw in down feathers of recently hatched chicks. There is limited data on chick toxicity to compare this value to, but Ackerman et al. (*17*) found that mercury concentrations (geometric mean ± SE) in down feathers of dead stilt chicks at hatching (16.43 ± 2.19 $\mu g\ g^{-1}$ fw) were higher than levels in randomly sampled live chicks of similar age (9.98 ± 0.77 $\mu g\ g^{-1}$ fw). Down feather mercury concentrations in live stilt chicks correspond to a predicted fresh egg concentration of 0.74 $\mu g\ g^{-1}$ fww, whereas down feathers from dead stilt chicks correspond to a predicted fresh egg concentration of 1.19 $\mu g\ g^{-1}$ fww (eq 5). These data suggest that although eggs with mercury concentrations above >1.0 $\mu g\ g^{-1}$ ww can still hatch, the residual effects of maternally derived mercury on early chick mortality may continue to impair overall reproduction. This is particularly important because it means that commonly used end points, such as egg hatchability, likely underestimate the risk of mercury contamination to avian reproduction. However, by using the equation we derived between mercury concentrations in down feathers and eggs, inclusion of early chick mortality into the egg exposure criterion allows us to integrate toxicity risk for two avian life-stages into a single tissue matrix.

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Literature Cited

(1) Thompson, D. R. Mercury in birds and terrestrial mammals. In *Environmental Contaminants in Wildlife, Interpreting Tissue Concentrations*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; CRC Press LCC: Boca Raton, FL, 1996; pp 341−356.

(2) Wiener, J. G.; Krabbenhoft, D. P.; Heinz, G. H.; Scheuhammer, A. M. Ecotoxicology of mercury. In *Handbook of Ecotoxicology*, 2nd ed.; Hoffman, D. J., Rattner, B. A., Burton, G. A., Jr., Cairns, J., Jr., Eds; CRC Press LCC: Boca Raton, FL, 2003; pp 409−463.

(3) Scheuhammer, A. M.; Meyer, M. W.; Sandheinrich, M. B.; Murray, M. W. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* **2007**, *36*, 12–18.

(4) Heath, J. A.; Frederick, P. C. Relationships among mercury concentrations, hormones, and nesting effort of white ibises (*Eudocimus albus*) in the Florida Everglades. *Auk* **2005**, *122*, 255–267.

(5) Evers, D. C.; Savoy, L. J.; DeSorbo, C. R.; Yates, D. E.; Hanson, W.; Taylor, K. M.; Siegel, L. S.; Cooley, J. H.; Bank, M. S.; Major, A.; Munney, K.; Mower, B. F.; Vogel, H. S.; Schoch, N.; Pokras, M.; Goodale, W. M.; Fair, J. Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* **2008**, *17*, 69–81.

(6) Evers, D. C.; Taylor, K. M.; Major, A.; Taylor, R. J.; Poppenga, R. H.; Scheuhammer, A. M. Common loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology* **2003**, *12*, 69–81.

(7) Albers, P. H.; Koterba, M. T.; Rossmann, R.; Link, W. A.; French, J. B.; Bennett, R. S.; Bauer, W. C. Effects of methylmercury on reproduction in American kestrels. *Environ. Toxicol. Chem.* **2007**, *26*, 1856–1866.

(8) Heinz, G. H.; Hoffman, D. J. Embryotoxic thresholds of mercury: Estimates from individual mallard eggs. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 257–264.

(9) Heinz, G. H.; Hoffman, D. J.; Klimstra, J. D.; Stebbins, K. R.; Konrad, S. L.; Erwin, C. A. Species differences in the sensitivity of avian embryos to methylmercury. *Arch. Environ. Contam. Toxicol.* **2008**, in press.

(10) Spalding, M. G.; Frederick, P. C.; McGill, H. C.; Bouton, S. N.; Richey, L. J.; Schumacher, I. M.; Blackmore, C. G. M.; Harrison, J. Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. *J. Wildl. Dis.* **2000**, *36*, 423–435.

(11) Kenow, K. P.; Grasman, K. A.; Hines, R. K.; Meyer, M. W.; Gendron-Fitzpatrick, A.; Spalding, M. G.; Gray, B. R. Effects of methylmercury exposure on the immune function of juvenile common loons (*Gavia immer*). *Environ. Toxicol. Chem.* **2007**, *26*, 1460–1469.

(12) Spalding, M. G.; Frederick, P. C.; McGill, H. C.; Bouton, S. N.; McDowell, L. R. Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. *J. Wildl. Dis.* **2000**, *36*, 411–422.

(13) Longcore, J. R.; Dineli, R.; Haines, T. A. Mercury and growth of tree swallows at Acadia National Park, and at Orono, Maine, USA. *Environ. Monit. Assess.* **2007**, *126*, 117–127.

(14) Meyer, M. W.; Evers, D. C.; Hartigan, J. J.; Rasmussen, P. S. Patterns of common loon (*Gavia immer*) mercury exposure, reproduction, and survival in Wisconsin, USA. *Environ. Toxicol. Chem.* **1998**, *17*, 184–190.

(15) Heinz, G. Effects of low dietary levels of methyl mercury on mallard reproduction. *Bull. Environ. Contam. Toxicol.* **1974**, *11*, 386–392.

(16) Finley, M. T.; Stendell, R. C. Survival and reproductive success of black ducks fed methyl mercury. *Environ. Pollut.* **1978**, *16*, 51–63.

(17) Ackerman, J. T.; Takekawa, J. Y.; Eagles-Smith, C. A.; Iverson, S. A. Mercury contamination and effects on survival of American avocet and black-necked stilt chicks in San Francisco Bay. *Ecotoxicology* **2008**, *17*, 103–116.

(18) Fimreite, N. Effects of dietary methlymercury on ring-necked pheasants. *Can. Wildl. Serv. Occas. Pap.* **1971**, *9*, 39 pp.

(19) Heinz, G. H. Methylmercury: Reproductive and behavioral effects on three generations of mallard ducks. *J.Wildl. Manage.* **1979**, *43*, 394–401.

(20) Kenow, K. P.; Gutreuter, S.; Hines, R. K.; Meyer, M. W.; Fournier, F.; Karasov, W. H. Effects of methyl mercury exposure on the growth of juvenile common loons. *Ecotoxicology* **2003**, *12*, 171–182.

(21) Monteiro, L. R.; Furness, R. W. Kinetics, dose-response, excretion, and toxicity of methylmercury in free-living Cory's shearwater chicks. *Environ. Toxicol. Chem.* **2001**, *20*, 1816–1823.

(22) Fournier, F.; Karasov, W. H.; Kenow, K. P.; Meyer, M. W.; Hines, R. K. The oral bioavailability and toxicokinetics of methylmercury in common loons (*Gavia immer*) chicks. *Comp.Biochem.Physiol. Part A* **2002**, *133*, 703–714.

(23) Kenow, K. P.; Meyer, M. W.; Hines, R. K.; Karasov, W. H. Distribution and accumulation of mercury in tissues of captive-reared common loon (*Gavia immer*) chicks. *Environ. Toxicol. Chem.* **2007b**, *26*, 1047–1055.

(24) Becker, P. H.; Furness, R. W.; Henning, D. Mercury dynamics in young common tern (*Sterna hirundo*) chicks from a polluted environment. *Ecotoxicology* **1993**, *2*, 33–40.

(25) Becker, P. H.; Henning, D.; Furness, R. W. Differences in mercury contamination and elimination during feather development in gull and tern broods. *Arch. Environ. Contam. Toxicol.* **1994**, *27*, 162–167.

(26) Ackerman, J. T.; Takekawa, J. Y.; Eagles-Smith, C. A.; Iverson, S. A. Survival of postfledging Forster's terns in relation to mercury exposure in San Francisco Bay. *Ecotoxicology* **2008**, *17*, 789–801.

(27) Hays, H.; LeCroy, M. Field criteria for determining incubation stage in eggs of the common tern. *Wilson Bull.* **1971**, *83*, 425–429.

(28) Robinson, J. A.; Oring, L. W.; Skorupa, J. P.; Boettcher, R., American avocet (*Recurvirostra americana*). In *The Birds of North America, No. 449*; Poole, A., Gill, F., Eds.; The Academy of Natural Sciences and The American Ornithologists' Union: Washington, DC, 1997.

(29) Robinson, J. A.; Reed, J. M.; Skorupa, J. P.; Oring, L. W. Black-necked stilt (*Himantopus mexicanus*). In *The Birds of North America, No. 449*; Poole, A., Gill, F., Eds.; The Academy of Natural Sciences and The American Ornithologists' Union: Washington, DC, 1999.

(30) McNicholl, M. K.; Lowther, P. E.; Hall, J. A. Forster's tern (Sterna forsteri). In *The Birds of North America, No. 595*; Poole, A., Gill, F., Eds.; The Academy of Natural Sciences and The American Ornithologists' Union: Washington, DC, 2001.

(31) Stebbins, K. R.; Klimstra, J. D.; Eagles-Smith, C. A.; Ackerman, J. T.; Heinz, G. H. A non-lethal micro-sampling technique to monitor the effects of mercury on wild bird eggs. **2009**, *Environ. Toxicol. Chem. 28*, 465–470.

(32) Becker, P. H. Egg mercury levels decline with the laying sequence in Charadriiformes. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 762–767.

(33) Thompson, D. R.; Furness, R. W. Comparison of the levels of total and organic mercury in seabird feathers. *Mar. Pollut. Bull.* **1989**, *20*, 577–579.

(34) Heinz, G. H.; Hoffman, D. J. Mercury accumulation and loss in mallard eggs. *Environ. Toxicol. Chem.* **2004**, *23*, 222–224.

(35) Evers, D. C.; Burgess, N. M.; Champoux, L.; Hoskins, B.; Major, A.; Goodale, W. M.; Taylor, R. J.; Poppenga, R.; Daigle, T. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology* **2005**, *14*, 193–221.

(36) Ackerman, J. T.; Eagles-Smith, C. A.; Takekawa, J. Y.; Demers, S. A.; Adelsbach, T. L.; Bluso, J. D.; Miles, A. K.; Warnock, N.; Suchanek, T. H.; Schwarzbach, S. E. Mercury concentrations and space use of pre-breeding American avocets and black-necked stilts in San Francisco Bay. *Sci. Total Environ.* **2007**, *384*, 452–466.

(37) Ackerman, J. T.; Eagles-Smith, C. A.; Takekawa, J. Y.; Bluso, J. D.; Adelsbach, T. L. Mercury concentrations in blood and feathers of pre-breeding Forster's terns in relation to space use of San Francisco Bay habitats. *Environ. Toxicol. Chem.* **2008**, *27*, 897–908.

(38) *U.S. EPA. Method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry, Test methods for evaluating solid waste, physical/chemical methods SW 846, Update IVA*; US Government Printing Office (GPO): Washington, DC, USA, 2000.

(39) Stickel, L. F.; Wiemeyer, S. N.; Blus, L. J. Pesticide residue in eggs of wild birds: Adjustments for loss of moisture and lipid. *Bull. Environ. Contam. Toxicol.* **1973**, *9*, 193–196.

(40) Hoyt, D. F. Practical methods of estimating volume and fresh weight of bird eggs. *Auk* **1979**, *96*, 73–77.

(41) Hill, E. F.; Henny, C. J.; Grove, R. A. Mercury and drought along the lower Carson River, Nevada: II. Snowy egret and black-crowned night-heron reproduction on Lahontan Reservoir, 1997−2006. *Ecotoxicology* **2008**, *17*, 117–131.

(42) Schwarzbach, S. E.; Albertson, J. D.; Thomas, C. M. Effects of predation, flooding, and contamination on reproductive success of California clapper rails (*Rallus longirostris* obsoletus) in San Francisco Bay. *Auk* **2006**, *123*, 45–60.
(43) Heinz, G. H.; Hoffman, D. J. Predicting mercury in mallard ducklings from mercury in chorioallantoic membranes. *Bull. Environ. Contam. Toxicol.* **2003**, *70*, 1242–1246.
(44) Cobb, G. P.; Norman, D. M.; Kendall, R. J. Organochlorine contaminant assessment in great blue herons using traditional and nonlethal monitoring techniques. *Environ. Pollut.* **1994**, *83*, 299–309.
(45) Bouton, S. N.; Frederick, P. C.; Spalding, M. G.; McGill, H. Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. *Environ. Toxicol. Chem.* **1999**, *18*, 1934–1939.
(46) Burgess, N. M.; Meyer, M. W. Methylmercury exposure associated with reduced productivity in common loons. *Ecotoxicology* **2008**, *17*, 83–91.

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