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Total and Methyl Mercury Concentrations in Seabird Feathers and Eggs

Alexander L. Bond · Antony W. Diamond

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Abstract Seabirds are used frequently as indicators of mercury contamination in marine ecosystems, but few studies have examined the forms of mercury found in seabird tissues. Here we compare concentrations of total and organic mercury in feathers ($n = 5$) of six sympatric nesting seabirds and in egg components of Leach's storm-petrels from Machias Seal Island, New Brunswick, Canada, during the 2006 breeding season. Essentially all (82–133%) mercury found in seabird feathers and egg components was methyl mercury, with no interspecific differences in percentage methyl mercury. This pattern is consistent with the hypothesis that feather-molt and egg production eliminate toxic methyl mercury, while inorganic forms from demethylation in the liver remain in internal tissues. Additional studies across more species, and comparisons with percentage methyl mercury in internal tissues, are required to validate this theory.

Mercury is a pervasive anthropogenic environmental contaminant that is transported atmospherically around the world (Nriagu and Pacyna 1988; Nriagu 1989; Mason and Sheu 2002) and is particularly elevated in the Gulf of Maine region (Evers and Clair 2005). Seabirds have been used frequently as indicators of mercury contamination in the marine environment (Burger 1993; Monteiro and Furness 1995; Furness and Camphuysen 1997; Kim et al. 1998; Burger and Gochfeld 2004; Goodale et al. 2008), yet few studies have examined the forms of mercury present in seabird tissues (Thompson and Furness 1989a, b; Kim et al. 1996; Burger and Gochfeld 2002). Methyl mercury represents usually less than 1% of the mercury in marine and freshwaters, yet since methyl mercury is the form that bioaccumulates in the food web, top predators contain methyl mercury levels of at least 95% of the total mercury (Weiner et al. 2003). Methyl mercury affects the nervous, circulatory, and endocrine systems in birds (Fimreite and Karstad 1971; Spalding et al. 2000; UNEP 2002), and it is considered to be mercury's most toxic form (UNEP 2002; Weiner et al. 2003). Effects of mercury contamination in birds are species-specific, as are effect levels (Burger 1993; Burger and Gochfeld 1997).

Some seabirds apparently tolerate higher total mercury concentrations than other birds, but this is based on assessments of total mercury in tissues such as feathers and whole eggs (e.g., Monteiro et al. 1999; Burger and Gochfeld 2000), and determining effect levels in seabirds is challenging, as many studies have focused on other families (e.g., quails and pheasants [Burger and Gochfeld 1997]). Although controlled dosing studies have been done in some seabirds (Monteiro and Furness 2001), these did not confirm effect levels, which are species-specific (Burger 1993). Only two previous studies (Thompson and Furness 1989b; Kim et al. 1996) have explicitly compared total and methyl mercury levels in adult seabirds. Feathers are easily collected, and representative of internal total mercury burden (Agusa et al. 2005).

Here we present comparisons of total and methyl mercury from body contour feathers of six seabird species from

A. L. Bond · A. W. Diamond
Atlantic Cooperative Wildlife Ecology Research Network & Department of Biology, University of New Brunswick, PO Box 45111, Fredericton, New Brunswick, Canada E3B 6E1

Present Address:
A. L. Bond (✉)
Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland & Labrador, Canada A1B 3X9
e-mail: abond@mun.ca

three families—Leach's storm-petrels *Oceanodroma leucorhoa* (Hydrobatidae), arctic *Sterna paradisaea* and common terns *S. hirundo* (Laridae), and Atlantic puffins *Fratercula arctica*, common murres *Uria aalge*, and razorbills *Alca torda* (Alcidae)—as well as from storm-petrel eggs, from New Brunswick, Canada.

Materials and Methods

Sample Collection and Preparation

All samples were collected on Machias Seal Island, Bay of Fundy, Canada (44°30'N, 67°06'W), during the 2006 breeding season. Breast feathers were collected from five individuals of unknown sex from each species during routine banding, or from freshly dead carcasses of birds that had collided with structures on the island. Feathers were stored at −18°C in individual sterile polythene bags until analysis. Immediately prior to analysis, feathers were washed for 1 min each in a 0.25 M NaOH solution and three deionized water baths to remove external contamination. Mercury in feathers is unlikely affected by such treatments (Appelquist et al. 1984).

Five fresh storm-petrel eggs were collected soon after laying, and freshness was determined by flotation. Eggs were wrapped individually in cellophane and frozen at −18°C for transportation. Albumin and yolk were separated manually and weighed (±0.1 g) before drying in a Virtis Benchtop freeze-dryer for 36–48 h.

Mercury Analysis

Total mercury concentration of the samples was determined by high-temperature combustion SP-3D analyzer (Nippon Instruments Corp., Japan). One whole feather was analyzed for both total and methyl mercury. After combustion at 850°C, mercury was converted catalytically to elemental mercury. Following dual gold amalgamation the quantity of mercury was measured by the cold vapor atomic absorption (CVAA) method at a wavelength of 253.7 nm. The samples required no chemical treatment prior to the analysis. Up to 10.0 mg of dried yolk and albumin samples from eggs and between 10 and 50 mg of feather samples were placed on a layer of an additive (mixture of sodium carbonate and calcium hydroxide; EMD Chemicals) in a ceramic boat as suggested by the supplier. The sample was then covered with a layer of the same additive. A layer of aluminum oxide (Al_2O_3; EM Science) was placed over the sodium carbonate-calcium hydroxide layer. Another layer of the latter covered this aluminum oxide layer and the boat was transferred manually into the ceramic thermal decomposition chamber. Reproducibility and accuracy of the method were assessed every five samples using a standard of 5.0 ppb. Dogfish muscle (DORM-2, NRC) was the standard reference material, with certified values of 4.64 ± 0.26 mg/kg dry weight. Recovery of DORM-2 was 98% ($n = 4$; values—4.51, 4.60, 4.56, and 4.47 mg/kg).

Determination of methyl mercury concentration was carried out by capillary gas chromatography coupled with atomic fluorescence spectrometry (GC-AFS). About 0.05 g of dried yolk and albumin samples and between 0.01 and 0.02 g of feather samples were placed in 20-ml scintillation vials with Teflon PTFE caps. Then 2.0 ml of deionized water and 2.0 ml of 6 M potassium hydroxide solution were added and the samples were shaken for 4 h at 300 rpm. After that, 2.0 ml of 6 M hydrochloric acid was added and the pH was checked to be <3.0 (high acidic medium). Four millimeters of an acidic (5% H_2SO_4, v/v) potassium bromide/1.0 M copper sulfate mixture was added. To extract MeHg into the organic phase, 5.0 ml of methylene chloride was added and the vials were shaken overnight at 300 rpm. The next day the samples were centrifuged for 10 min at 3500 rpm. A 2.0-ml aliquot of the methylene chloride was transferred to 7-ml glass tubes, and 1.0 ml of 0.01 M sodium thiosulfate added to each sample. The samples were shaken for 20 min, mixed on a vortex mixer, and centrifuged for 5 min at 3500 rpm. A volume of 0.4 ml of the aqueous top layer (sodium thiosulfate) was placed in polyethylene microcentrifuge vials, and 0.3 ml of an acidic potassium bromide/1.0 M copper sulfate mixture (3:1) and 0.3 ml of dichloromethane were added. Again, the vials were shaken for 15 min, mixed, and centrifuged. Finally, the lower phase (dichloromethane containing the extracted MeHg) was extracted carefully and transferred through a small layer of anhydrous sodium sulfate (packed in a pipette tip) to a 2-ml amber glass vial with a 200-µl glass insert. Methyl and total mercury were determined for the same feather samples simultaneously. Two analytical blanks and a certified reference material (DORM-2, NRC, 4.47 ± 0.32 mg kg^{-1} methyl mercury) were run every five samples to evaluate reproducibility and accuracy of analysis method. Methyl mercury recovery from DORM-2 was 94% ($n = 4$; values—4.46, 4.15, 4.03, and 4.21 mg/kg) and all feather samples were run in duplicate, with mean standard deviations within samples of <0.05 ppm.

Statistical Methods

Statistical tests were performed in SPSS v.11 using a significance level of 0.05 for all tests. We used the Games-Howell post hoc test (GH text [Games and Howell 1976]) to make pairwise comparisons among species following analysis of variance (ANOVA) testing. We chose the GH

test since it is relatively robust to comparisons involving fewer than eight groups, with unequal variances, but with $n \geq 5$ in each group, and because we were not necessarily interested in the level of significance but desired greater power than Dunnett's T3 or C procedures (Day and Quinn 1989).

Outliers in linear regression models were detected using Cook's distance (D_i), with points deemed outliers if $D_i > F_{0.50}$ with n and $n - k$ degrees of freedom, where n is the sample size and k is the number of coefficients in the regression analysis (Cook 1977, 1979). Regression models of methyl and total mercury were done to determine the percentage methyl mercury as indicated by the slope.

Mercury concentrations are presented below as mean ± SD parts per billion (ppb, or μg kg^{-1}) fresh weight for feathers and mean ± SD parts per billion dry weight for egg components.

Results

Feathers

There was no difference among species in the mean proportion of methyl mercury in seabird feathers (ANOVA, $F_{5,24} = 0.92$, $p = 0.49$). Methyl mercury levels were significantly correlated with total mercury concentrations among species ($r = 0.912$, $p < 0.001$). The slope of the regression of total against methyl mercury, representing the percentage methyl mercury (β), was 1.06 (Fig. 1a). Arctic tern ($r = 0.96$, $p = 0.01$, $\beta = 0.81$) and Leach's storm-petrels ($r = 0.92$, $p = 0.03$, $\beta = 1.10$; Fig. 1b, c) had a significant relationship when species were examined individually. No outliers were detected (all D_i's < 0.38).

The proportion of methyl mercury ranged from 82% in razorbills to 133% in common murres, averaging 104.0% ± 41.2% across all species (Table 1; note that values >100% arise from differences in mercury concentrations within the tissue). Since there is spatial heterogeneity in the tissues, duplicate samples do vary, and this is not due to machine variability. There were significant differences in the amount of methyl mercury among species (ANOVA, $F_{5,\ 24} = 5.41$, $p = 0.002$) but post hoc tests could not detect this difference (GH test, all p's > 0.20). Variation in feather methyl mercury concentration between the two samples from each individual was small, as measured by the coefficient of variation (range, 0.34–11.34%; overall mean, 3.58% ± 2.92%) and was similar across species (ANOVA, $F_{5,\ 24} = 0.26$, $p = 0.93$). There was no relationship between the total mercury concentration and the proportion of methyl mercury ($r = -0.021$, $p = 0.91$).

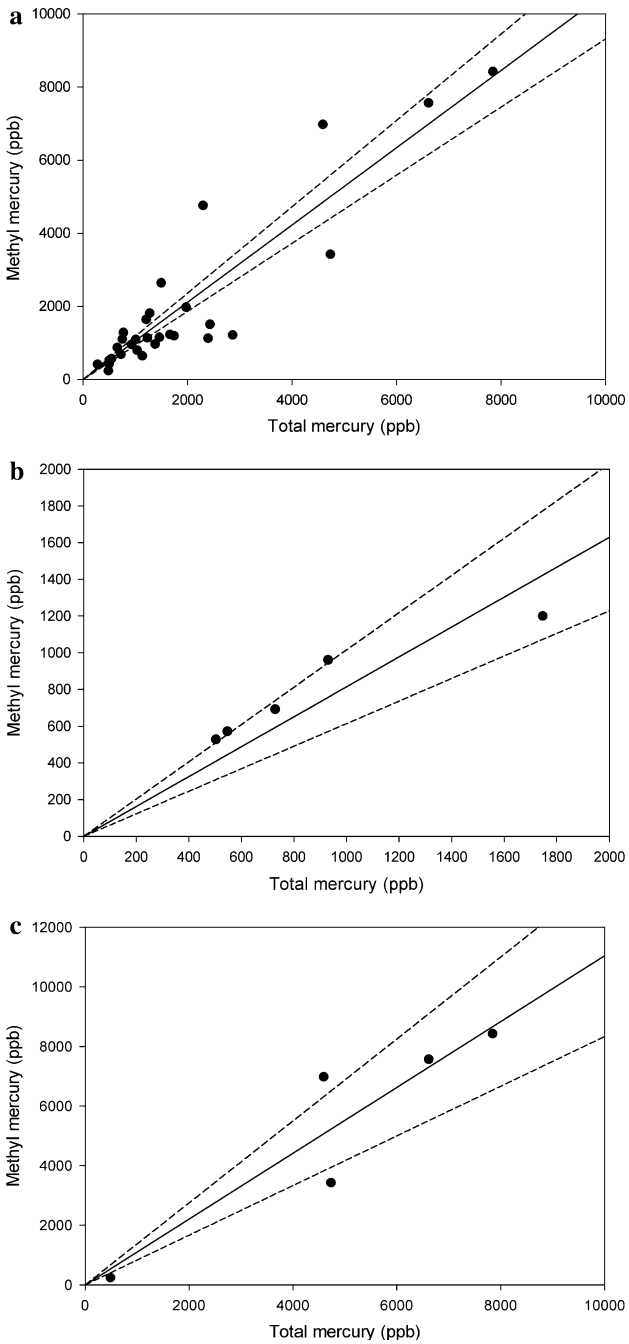

Fig. 1 Linear regression of feather mercury and methyl mercury concentrations with 95% confidence intervals from (**a**) six seabird species ($n = 30$), (**b**) arctic tern ($n = 5$), and (**c**) Leach's storm-petrel ($n = 5$) from Machias Seal Island in 2006

Albumin

Albumin methyl mercury concentrations ranged from 3836 to 5178 ppb (mean, 4569 ± 579 ppb). Total mercury ranged from 3849 to 7994 ppm (mean, 5502 ± 1535 ppb), of which 51.5%–107.2% (mean, 87.6% ± 21.7%) was methyl mercury. Cook's distance identified two outliers from the

Table 1 Total and organic mercury concentrations (parts per billion, fresh weight) in seabird breast feathers from Machias Seal Island, NB, in 2006

Species	*n*	Mean total mercury, ppb ± SD	Mean methyl mercury, ppb ± SD	Mean methyl mercury, % of total ± SD
Arctic tern	5	891 ± 507	791 ± 284	95 ± 15
Common murre	5	987 ± 361	1249 ± 349	133 ± 33
Common tern	5	1380 ± 991	1619 ± 1814	114 ± 65
Razorbill	5	1404 ± 559	1073 ± 383	82 ± 35
Atlantic puffin	5	1805 ± 668	1634 ± 664	100 ± 50
Leach's storm-petrel	5	4855 ± 2791	5330 ± 3423	99 ± 40

Note: Methyl mercury values are means of duplicate samples

albumin data set ($D_i = 1.38, 5.93$; Table 2), but even when these are removed, the regression model is not significant ($r = 0.85$, $F_{1,1} = 2.64$, $p = 0.35$), and the sample size was severely reduced, so they were retained in the data set. When included, the regression model is not significant ($r = 0.11$, $F_{1,3} = 0.38$, $p = 0.86$; Fig. 2).

Yolk

Yolk methyl mercury concentrations were significantly lower than those in albumin (paired-sample *t*-test, $t_4 = 16.29$, $p < 0.001$), and concentrations between yolk and albumin were not correlated ($r = -0.15, p = 0.81$), even when outliers were removed ($r = 0.41$, $p = 0.59$). Methyl mercury values from yolk ranged from 128 to 349 ppb (mean, 227 ± 83 ppb), and total mercury ranged from 192 to 404 ppb (mean, 298 ± 81). Methyl mercury represented 67–86% (mean, 75% ± 7.3%) of the total mercury concentration (Table 2). Cook's distance identified one outlier ($D_i = 2.37$; Table 2), but the regression model of percentage methyl mercury and total mercury was significant whether this was excluded ($r = 0.99$, $F_{1,2} = 476.57$, $p = 0.002$) or included ($r = 0.99$, $F_{1,3} = 149.98$, $p = 0.001$; Fig. 2). The proportion of methyl mercury was significantly higher in albumin than yolk when outliers were removed (paired-sample *t*-test, $t_3 = 9.04$, $p = 0.003$; Table 2).

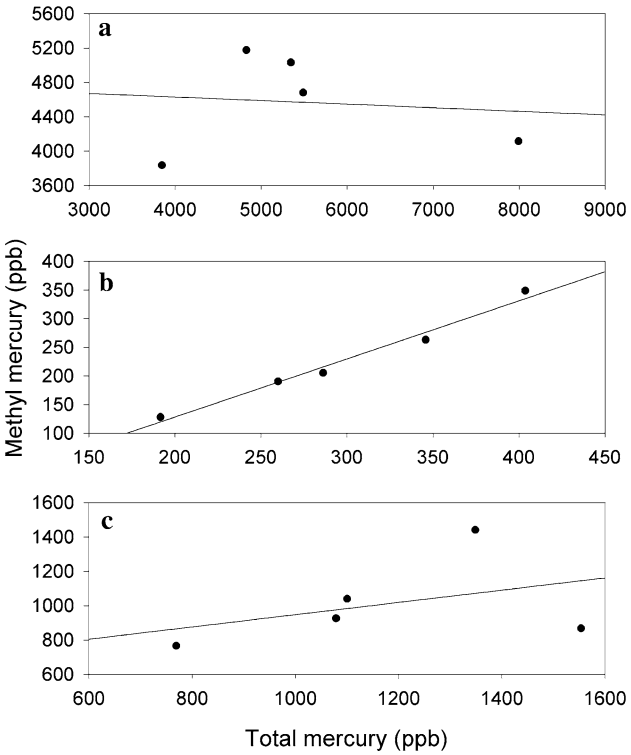

Fig. 2 Linear regression of (**a**) albumin, (**b**) yolk, and (**c**) whole-egg methyl and total mercury concentrations from Leach's storm-petrels on Machias Seal Island in 2006 ($n = 5$)

Table 2 Total and methyl mercury concentrations (parts per billion, dry weight) in albumin, yolk, and whole eggs of Leach's storm-petrels on Machias Seal Island, NB, in 2006

Sample No.	Albumin			Yolk			Whole egg		
	THg (ppb)	MeHg (ppb)	% MeHg	THg (ppb)	MeHg (ppb)	% MeHg	THg (ppb)	MeHg (ppb)	% MeHg
1	3849	3836[a]	100	260	191	73.3	769	767	100
2	4829	5178	107	346	263	76.1	1349	1441	107
3	5347	5033	94	286	205	71.7	1100	1039	94
4	5490	4682	85	192	128	66.9	1079	926	86
5	7994	4117[a]	52	404	349[a]	86.4	1554	868	56
Mean ± SD	5501 ± 1535	4569 ± 579	88 ± 22	298 ± 81	227 ± 83	75 ± 7	1170 ± 297	1008 ± 291	89 ± 20

[a] Outlier as determined by Cook's distance (see text for explanation)

Whole Eggs

Whole-egg total mercury concentrations (1170 ± 297 ppb) were 89% ± 20% methyl mercury (1008 ± 261 ppb). The regression model of total against methyl mercury was not significant ($r = 0.41$, $F_{1,3} = 0.59$, $p = 0.50$), although much of the variation in percentage methyl mercury was driven by one sample (56%; all others, 85–107%), and no outliers were detected in whole-egg total or methyl mercury concentrations (all D_i's < 0.52). When the one consistent outlier for albumin and yolk is removed, the model is almost significant ($r = 0.94$, $F_{1,2} = 15.49$, $p = 0.06$; Fig. 2c).

Discussion

Essentially all mercury in seabird feathers is organic methyl mercury, and deviations from this are likely caused by heterogeneity within the tissue. Leach's storm-petrels have higher mercury concentrations in feathers, eggs, and blood than other seabirds in the Gulf of Maine (Bond 2007; Goodale et al. 2008), and this could be related to either phylogeny, lengthy molt cycles (Bond 2007), or high mercury concentration in myctophid fish, their main prey items (Martins et al. 2006). The levels observed in feathers from this study are below those thought to cause sublethal effects (Burger and Gochfeld 2000), but this level has not been confirmed in laboratory studies of the species we sampled.

Feather molt and growth is the main mercury excretion pathway for seabirds (Braune and Gaskin 1987; Monteiro and Furness 2001), and it has been hypothesized that some seabirds have the ability to demethylate organic mercury, creating the less toxic inorganic form (Thompson and Furness 1989a; Kim et al. 1996). Inorganic mercury is immobile and remains in the liver, while the toxic form is excreted during feather molt. Thus, mercury concentrations in feathers could act as an indicator of the efficiency of demethylation, assuming that individuals share a common mercury intake. It would then follow that species that have a poor demethylation ability would have higher mercury concentrations in feathers as an alternative method for eliminating methyl mercury. While examining mercury concentrations in internal tissues would be ideal for determining efficiency of demethylation (Monteiro and Furness 2001), this type of destructive sampling may not be appropriate in all cases.

When this was tested using the seven seabirds examined by Kim et al. (1996)—royal *Diomedea epomophora*, black-footed *Phoebastria nigripes* and Laysan albatrosses *P. immutabilis*, white-chinned petrels *Procellaria aequinoctialis*, northern fulmars *Fulmarus glacialis*, brown boobies *Sula leucogaster*, herring gulls *Larus argentatus*, and arctic terns—the relationship between mean liver percentage methyl mercury and mean feather total mercury was almost significant ($F_{1,6} = 4.36$, $p = 0.08$); however, this study compared only seven species. Further investigation of the link between liver and feather mercury types is needed before firm conclusions can be drawn.

Albumin total mercury concentrations were higher than those in yolk, as mercury is bound to protein rather than lipid and egg protein is concentrated in albumin (Magat and Sell 1979). This is the first study to document the differences in mercury types in egg components. Methyl mercury made up the majority of the mercury concentration in albumin from Leach's storm-petrels, and significantly less so in yolk. Again, this is likely due to the increased protein content of albumin over yolk egg fractions. Examinations of additional species are required. As with feathers, it appears that the mercury forms are excreted differentially according to the inherent chemical properties of each compound, although we did not explicitly test internal organs for body mercury burden.

Conclusions

The vast majority of mercury present in seabird feathers and eggs is in the methyl form. This appears to be the result of differential excretion of toxic forms of mercury, while less toxic forms are sequestered in internal tissues. This pattern requires further corroboration across more species before generalizations regarding seabird mercury toxicology can be made.

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