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Avian mercury exposure and toxicological risk across western North America: A synthesis

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Abstract

Methylmercury contamination of the environment is an important issue globally and birds are useful bioindicators for mercury monitoring programs. The available data on mercury contamination of birds in western North America were synthesized. Original data from multiple databases were obtained and a literature review was conducted to obtain additional mercury concentrations. In total, 29219 original bird mercury concentrations from 225 species were compiled, and an additional 1712 mean mercury concentrations, representing 19998 individuals and 176 species, from 200 publications were obtained. To make mercury data comparable across bird tissues, published equations of tissue mercury correlations were used to convert all mercury concentrations into blood-equivalent mercury concentrations. Blood-equivalent mercury concentrations differed among species, foraging guilds, habitat types, locations, and ecoregions. Piscivores and carnivores exhibited the greatest mercury concentrations, whereas herbivores and granivores exhibited the lowest mercury concentrations. Bird mercury concentrations were greatest in ocean and salt marsh habitats and lowest in terrestrial habitats. Bird mercury concentrations were above toxicity benchmarks in many areas throughout western North America, and multiple hotspots were identified. Additionally, published toxicity benchmarks established in multiple tissues were summarized and translated into a common blood-equivalent mercury concentration. Overall, 66% of birds sampled in western North American exceeded a blood-equivalent mercury concentration of $0.2 \,\mu g/g$ wet weight (ww; above background levels), which is the lowest-observed

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effect level, 28% exceeded 1.0 μ g/g ww (moderate risk), 8% exceeded 3.0 μ g/g ww (high risk), and 4% exceeded 4.0 μ g/g ww (severe risk). Mercury monitoring programs should sample bird tissues, such as adult blood and eggs, that are most-easily translated into tissues with well-developed toxicity benchmarks and that are directly relevant to bird reproduction. Results indicate that mercury contamination of birds is prevalent in many areas throughout western North America, and large-scale ecological attributes are important factors influencing bird mercury concentrations.

Keywords

Birds; Mercury; Eggs; Bioaccumulation; Toxicity Benchmarks

1. Introduction

Methylmercury contamination of the environment is an important issue globally because of continued anthropogenic emissions of mercury over time (Driscoll et al., 2013; Eagles-Smith et al., submitted to this issue; Weiss-Penzias et al., submitted to this issue), its ability to biomagnify through (primarily) aquatic food chains (Wiener et al., 2003), and its documented negative effects on fish and wildlife (Scheuhammer et al., 2007; Wiener et al., 2003). Birds are ubiquitous, top predators in many aquatic and terrestrial habitats, and often are subjected to elevated methylmercury concentrations (Cristol et al., 2008; Eagles-Smith et al., 2009a). Bird reproduction is particularly sensitive to mercury toxicity, with numerous documented deleterious effects to bird health, condition, behavior, and productivity (Table 1; Scheuhammer et al., 2007; Wiener et al., 2003). Together, these characteristics make birds useful bioindicators for local mercury contamination and regional monitoring programs (Day et al., 2012; Evers et al., 2011; Monteiro and Furness, 1995; Provencher et al., 2014; Weseloh et al., 2011).

Large-scale assessments of environmental pollution can be helpful for understanding the major drivers and distributions of contaminants in animals. A few studies have synthesized the available data on bird mercury contamination within the Great Lakes and northeastern regions of the United States and Canada (Evers et al., 2011; Jackson et al., 2015) and the Canadian Arctic (Mallory and Braune, 2012), but no such studies exist elsewhere in North America. Western North America is characterized by a diverse gradient of habitats, including both extremely dry and wet regions (National Assessment Synthesis Team, 2001). In particular, ephemeral wetland habitats are common across western North America (Tiner, 1984). The temporary wetting and drying of wetland habitats is often associated with biogeochemical conditions that tend to promote the methylation of inorganic mercury to its more toxic form, methylmercury (Ullrich et al., 2001). These ephemeral wetlands also tend to be highly productive and are greatly utilized by birds as foraging habitat (Murkin et al., 1997; Niemuth et al., 2006; Skagen et al., 2008). In addition to habitat-specific effects, mercury contamination in birds typically differs among foraging guilds, trophic levels, and species (Anderson et al., 2009; Blévin et al., 2013; Eagles-Smith et al., 2009a). Examining these effects over a large geographic area may identify hotspots of methylmercury contamination within bird populations, aid in prioritizing contaminant monitoring programs (Mason et al., 2005), and focus policy-making decisions.

In this synthesis paper, the available data on mercury contamination of birds in western North America are summarized. To do so, original, raw data from multiple databases were obtained and the literature was reviewed (published articles and reports) to extract mean mercury concentrations in birds for each species and site that has been studied. In total, nearly 30000 original, individual bird mercury concentrations from 225 species were compiled, and an additional >1700 mean mercury concentrations, representing nearly 20000 individuals and 176 species, from 200 publications were obtained. The goals were to describe the distribution of bird mercury contamination in western North America, identify potential hotspots, and examine the major factors influencing bird mercury concentrations. Specifically, the influence of species, foraging guild, habitat, ecoregion, and location on mercury contamination were examined for western North American birds. Additionally, the literature was reviewed, published toxicity benchmarks were summarized, and toxicity benchmarks established in multiple bird tissues were translated into a common bloodequivalent mercury concentration to integrate toxicity risk across avian life-stages and tissues. These toxicity benchmarks were then used to assess the toxicological risk of mercury exposure to birds in western North America.

2. Material and methods

2.1. Data acquisition: original data

Original data on mercury concentrations in individual birds from several sources were obtained. The U.S. Fish and Wildlife Service's Environmental Contaminants Data Management System (ECDMS) database (retrieved August 27, 2013), which contributed 25% of the data points, is an online database that houses contaminant data collected by government agencies. Additional original data were obtained from the authors' unpublished datasets at the U.S. Geological Survey (61% of the data); Biodiversity Research Institute (12%); the multi-partner Seabird Tissue and Archival Monitoring Project (STAMP; 2%); and Environment Canada (<1%). The databases were then merged, data was reviewed for quality, and the following information was extracted : bird species, tissue type (egg, whole blood, muscle, liver, kidney, and feathers), location (latitude and longitude), year, total mercury or methylmercury concentration, and units of measurement (including if data were reported in wet weight or dry weight). When location data were not reported within the study, study site descriptions (e.g., county or lake names) were used to assign approximate latitudes and longitudes using Google Earth[™]. Any incomplete data, including studies whose locations could not be determined, were excluded.

2.2. Data acquisition: literature review

A thorough literature review of all peer-reviewed journal articles and published reports documenting mercury concentrations in birds in western North America was conducted. Literature searches were conducted in Web of ScienceTM and Google ScholarTM. For each study, the following information was extracted: bird species, tissue type, location (latitude and longitude), year, mean mercury concentration, units of measurement (including if data were reported in wet weight or dry weight), and sample size. Sometimes, year was reported as a range and, in these cases, the midpoint was used. When year was not reported, the publication year minus one was applied. Similarly, when sample sizes were reported as

ranges, the midpoint was used as the sample size. When composite samples were used in a study, the number of composite samples was multiplied by the number of individual samples within the composites to calculate the effective sample size that was used in the study to produce the grand mean. When mean mercury concentrations were obtainable only from figures, rather than as values in a table or the text, the mean mercury concentration was visually approximated within the figure. Within the same study, mean mercury concentrations were kept separate for each species and location when possible; this often resulted with a single publication contributing multiple mean mercury concentrations, one mean for each species and location within the study. When location data were not reported within the study, study site descriptions (e.g., county and lake names) were used to assign approximate latitudes and longitudes using Google Earth[™].

2.3. Assigning bird taxonomy, foraging guilds, and habitats

For both the original and literature-review data, each species was assigned to a foraging guild and general habitat type. Taxonomy was based on the seventh edition of the American Ornithologists' Union's Checklist of North and Middle American Birds (retrieved Aug ust13, 2013 from http://checklist.aou.org/). Bird species were assigned to foraging guilds following DeGraaf et al. (1985) with the following modifications: (1) when a bird species occurred in multiple foraging guilds, such as piscivore and crustaceovore for several coastal seabirds, the primary foraging guild was used, and (2) when foraging guild differed by season (breeding, non-breeding, or year round), the foraging guild for the breeding season was used because most of the mercury data were from eggs or adults during the breeding season. Foraging guilds were categorized as piscivore, carnivore, insectivore, crustaceovore, molluscovore, vermivore, omnivore, granivore, or herbivore. Bird species were assigned to the following general habitats: ocean, coastal, salt marsh, both fresh and brackish water, freshwater, terrestrial-canopy, terrestrial-lower canopy, and terrestrial-ground. Habitats were assigned using DeGraaf et al.'s (1985) classifications as well as the Birds of North America series' (http://bna.birds.cornell.edu/bna/) habitat descriptions. All avian taxa (including order, family, and species), foraging guilds, and habitats are summarized in Table S1.

2.4. GIS data layers

Geographic Information Systems (GIS; ArcGIS 10.2, Environmental Research Systems Institute, Redlands, CA, USA) were used to attribute each sample location with landscape variables, including ecoregion and 100-km \times 100-km grid cell. The U.S. Environmental Protection Agency's ecoregion level one category (Commission for Environmental Cooperation, 1997), which separates North America into 15 distinct ecoregions, was used and two additional categories were added: one for samples collected in the Pacific and Arctic Oceans (including various small islands and atolls), and one for samples collected on the Hawaiian Islands, for a total of 17 possible ecoregions. The Create Fishnet geoprocessing tool (ArcGIS 10.2) was used to create a grid of cells, each measuring 100 km \times 100 km, across the extent of the sample locations in western North America, and then the Spatial Join geoprocessing tool (ArcGIS 10.2) was used to attribute each data point with the ecoregion and grid cell it occupied. Distribution maps of mercury concentrations in birds throughout western North America were produced using ArcGIS 10.2, and overlaid on a physical base layer provided by the U.S. National Park Service.

2.5. Data transformations and assumptions

Numerous data assumptions and transformations were necessary to consolidate, organize, and convert various tissue types and concentration units into similar values. First, only the following tissues were included : whole blood, eggs, muscle, liver, kidney, and fully grown feathers. These tissues represented >98% of the available data, and they are more readily comparable to one-another than the other available tissues, such as whole carcass. Second, data from any laboratory dosing or artificial studies that did not represent data from wild birds were excluded. Data from hazard assessments were included, because the impetus for a large number of studies was a known or suspected hazard (especially within the ECDMS dataset). Third, data were included only for eggs, adult tissues, or post-fledged juveniles. Samples collected from pre-fledged juveniles were excluded, because chicks undergo rapid changes in mercury concentrations in internal tissues as they grow and age (Ackerman et al., 2011) making any comparisons difficult. Fourth, data for both total mercury and methylmercury were included. All mercury in eggs (Ackerman et al., 2013), whole blood (Rimmer et al., 2005), muscle (Scheuhammer et al., 1998), and feathers (Thompson and Furness, 1989) was assumed to be in the methylmercury form, and, therefore, total mercury and methylmercury concentrations were used to represent methylmercury concentrations in birds. A significant proportion of the mercury in liver and kidney can be in the inorganic form (Eagles-Smith et al., 2009b; Scheuhammer et al., 1998; Thompson and Furness, 1989). Very few data (<1%) were available for these tissues as methylmercury concentrations, but, for those limited data, methylmercury concentrations were transformed into equivalent total mercury (THg) concentrations by using an adjustment of 88% of THg being in the methyl mercury form in liver (Eagles-Smith et al., 2009b). This assumption was justified because most data occurred below the 8.5 μ g/g dry weight (dw) liver threshold where demethylation begins, above which a smaller proportion of THg as methylmercury would be expected in the liver (Eagles-Smith et al., 2009b). No adjustments were necessary for methylmercury concentrations in kidney, because THg concentrations were always available when methylmercury concentrations in kidneys were reported. Fifth, to make the mercury data comparable across bird tissues, all tissue concentrations were converted into bloodequivalent THg concentrations $(\mu g/g)$ in wet weight (ww) using multiple equations from Eagles-Smith et al. (2008) and Ackerman et al. (2016a) detailed below. Before using these equations, it was necessary to convert THg concentration data from each tissue compartment into the same units, and thus all muscle, liver, kidney, and feather data were converted into dry weight THg concentrations ($\mu g/g dw$) using the reported percent moisture in the sample. Likewise, blood data were converted into wet weight THg concentrations ($\mu g/g$ ww) using the reported percent moisture in the sample in the few instances (<1%) where blood was reported in dry weight. When moisture content was not reported, an average moisture content of 79% in blood, 67% in liver, 70% in muscle, and 74% in kidney was used (Eagles-Smith et al., 2008). For eggs, it is important to report mercury concentrations on a fresh wet weight (fww) basis (Ackerman et al., 2013; Stickel et al., 1973); however, the necessary egg morphometrics to make these adjustments were not available in many of the raw datasets and this made the conversion to fresh wet weight not possible. Therefore, when egg morphometric data were unavailable, egg THg concentrations ($\mu g/g$) were converted on a dry weight basis into a wet weight basis using the reported percent moisture in the individual egg or, when moisture content was not reported, an average egg moisture content of 75%

was used (Ackerman et al., 2013). When egg morphometric data were available (i.e., authors' data), THg concentrations on a fresh wet weight basis (μ g/g fww) were used and calculated following Ackerman et al.(2013). In one instance, only albumen THg concentrations (μ g/g ww) were reported, and the albumen THg concentration was converted into a whole-egg THg concentration (μ g/g fww) using the predictive equation in Stebbins et al. (2009), before conversion into a blood-equivalent THg concentration. Hereafter, all egg THg concentrations are reported as simply μ g/g ww. For most analyses, data points that were derived from the same bird, but in a different tissue, were excluded. Priority was given to tissues from the same bird in the following order: whole blood, eggs, muscle, liver, kidney, and fully grown feathers (see Table 2).

To convert THg concentrations in bird tissues into THg concentrations in blood, the following equations (eqs. 1–4) from Eagles-Smith et al. (2008), which were developed from >600 birds of 4 bird species with a broad range of tissue THg concentrations, were used. For the feather equation, the predictive equation for breast feathers, rather than head feathers, was used because most of the feathers sampled are typically body feathers and this differentiation among feather types was not usually reported.

$$(R^2=0.90):\ln\left(Blood\ THg_{\frac{\mu g}{g}ww}\right) = 1.080 \times \ln\left(Bird\ Muscle\ THg_{\frac{\mu g}{g}dw}\right) - 1.024 \quad \text{eq. 1}$$

$$(R^{2}=0.88):\ln\left(Blood\ THg_{\frac{\mu g}{g}ww}\right)=0.970\times\ln\left(Bird\ Liver\ THg_{\frac{\mu g}{g}dw}\right)-1.929$$
eq. 2

$$(R^2=0.87):\ln\left(Blood\ THg_{\frac{\mu g}{g}ww}\right)=1.003\times\ln\left(Bird\ Kidney\ THg_{\frac{\mu g}{g}dw}\right)-2.008$$
 eq. 3

$$(R^2=0.32):\ln\left(Blood\ THg_{\frac{\mu g}{g}ww}
ight)=0.673\times\ln\left(Bird\ Feather\ THg_{\frac{\mu g}{g}dw}
ight)-1.673$$
eq. 4

To convert THg concentrations in eggs into equivalent THg concentrations in blood, the following equation (eq. 5) from Ackerman et al. (2016a), that was developed using 83 females and their full clutches for 3 species with a broad range of tissue THg concentrations, was used:

$$(R^2=0.95):\ln\left(Female\ Bird\ Blood\ THg_{\frac{\mu g}{g}ww}\right)=1.0734\times\ln\left(Egg\ THg_{\frac{\mu g}{g}fww}\right)+0.8149$$
eq. 5

These tissue conversion equations (1-5) were developed for multiple species and used the largest sample sizes currently available, and therefore represent the best available conversion

2.6. Statistical analysis

Linear mixed-effects models were used to examine factors influencing blood-equivalent THg concentrations in birds. Separate analyses were conducted for the two types of datasets: original raw data and the literature-review data. This separation ensured that data were not pseudoreplicated within the analyses because some of the original raw datasets were used to publish journal articles and reports that were summarized in the literature review dataset. THg concentrations in birds were log_e-transformed (natural log denoted as ln in equations) to improve normality. Back-transformed least squares means are reported with standard errors that were estimated using the delta method (Seber, 1982).

For the original raw dataset, \log_{e} -transformed blood-equivalent THg concentration was the dependent variable; foraging guild (9 guilds), habitat (8 habitats), and ecoregion (11 ecoregions) were fixed factors; and grid cell (432 grid cells each 100 km × 100 km), year (29 years: 1982–2015), and species (225 species) were random factors. To compare THg concentrations among species without the inclusion of habitat and foraging guild, a separate analysis was conducted where \log_{e} -transformed blood-equivalent THg concentration was the dependent variable; species was a fixed factor ; and grid and year were random factors. To examine the spatial distribution of THg in birds without the inclusion of habitat and foraging guild, an additional analysis was conducted where \log_{e} -transformed blood-equivalent THg concentration was the dependent variable; grid was a fixed factor; and species and year were random factors. This same analysis was repeated for each guild with sample sizes >5000 (within a guild) to specifically examine the distribution of THg in birds in the piscivore (*n*=10243), insectivore (*n*=8464), and omnivore (*n*=6685) guilds.

For the literature-review dataset, log_e-transformed mean blood-equivalent THg concentration was the dependent variable; foraging guild (8 guilds), habitat (8 habitats), and ecoregion (15 ecoregions) were fixed factors; and grid cell (313 grid cells), year (46 years: 1968–2013), and species (176 species) were random factors. For this analysis, blood-equivalent mean THg concentrations were weighted by the square-root of the study's effective sample size (i.e., the number of individuals used to estimate the mean), which placed more emphasis on the mean estimates that were derived from larger sample sizes. To compare mean THg concentrations among species without the inclusion of habitat and foraging guild, an analysis was conducted where log_e-transformed mean blood-equivalent THg concentrations in birds without the inclusion of habitat and foraging guild, an additional analysis was conducted where log_e-transformed mean blood-equivalent variable; grid was a fixed factor; and grid and year were random factors.

2.7. Literature review of mercury toxicity to birds and translation of toxicity benchmarks into a common blood-equivalent tissue

A thorough literature review was conducted and published toxicity benchmarks for all bird tissues were summarized (Table 1). These toxicity benchmarks were then integrated across avian tissues and life-stages into a single toxicity benchmark based on blood-equivalent THg concentrations. To do so, equations and assumptions noted in Table 1's footnotes were used to convert each of the toxicity benchmarks in various tissues into blood-equivalent THg concentrations. These equations and assumptions are described in more detail in section 2.5.

3. Results& Discussion

Original, raw data on THg concentrations in 29219 samples were obtained for 225 bird species. Most of the available data were for eggs (69%), followed by blood (16%), liver (7%), feathers (3%), kidney (3%), and muscle (2%). For most analyses, 1590 data points that were derived from the same bird, but in a different tissue, were excluded yielding a final sample size of 27629 birds. THg concentrations are summarized by species and tissues in Tables S2–S8. From the literature, 1712 mean THg concentrations were obtained for 176 bird species, representing 19998 individuals, from 200 publications (Supplementary Material: References). Figure 1a displays the distribution of THg concentrations using the original, raw data and Figure 1b displays the distribution of mean THg concentrations using data from the literature review.

3.1. Factors influencing bird mercury: original raw data

Bird blood-equivalent THg concentrations differed among foraging guilds ($F_{8,192.3}=11.72$, p<0.0001; Figure 2a) and habitat types ($F_{7,349.1}=12.69$, p<0.0001; Figure 2b), but did not differ among ecoregions ($F_{10,949.1}=0.93$, p=0.50). Piscivores ($0.33\pm0.05 \ \mu g/g \ ww$) and carnivores ($0.32\pm0.10 \ \mu g/g \ ww$) exhibited the greatest blood-equivalent least squares mean THg concentrations, whereas herbivores ($0.03\pm0.01 \ \mu g/g \ ww$) and granivores ($0.02\pm0.01 \ \mu g/g \ ww$) exhibited the lowest blood-equivalent least squares mean THg concentrations. These results are consistent with other studies that have found that birds foraging at higher trophic levels often have higher THg concentrations due to the biomagnification of methylmercury through food chains (Anderson et al., 2009; Blévin et al., 2013). In contrast, birds foraging on plants and seeds at the base of the food chain had substantially lower THg concentrations. Although these results were expected based on the ability of methylmercury to biomagnify, this is the first study to demonstrate differences in THg concentrations among such a wide range of foraging guilds.

Bird blood-equivalent least squares mean THg concentrations were greatest in ocean $(0.49\pm0.22 \ \mu\text{g/g ww})$ and salt marsh $(0.31\pm0.07 \ \mu\text{g/g ww})$ habitats and lowest in terrestrialground habitats $(0.04\pm0.01 \ \mu\text{g/g ww})$; Figure 2b). Aquatic environments have biogeochemical conditions that are more conducive to methylation and methylmercury is more prevalent in aquatic than terrestrial ecosystems (Ullrich et al., 2001); therefore, it was not surprising that THg concentrations in birds would be lower in terrestrial than aquatic environments. However, some terrestrial birds can receive substantial aquatic subsidies of methylmercury through emergent aquatic insects and the associated food web (Cristol et al.,

2008; Jackson et al., 2011b), so some terrestrial species can be exposed to higher methylmercury levels than would be assumed based upon their terrestrial foraging habits. Ocean and estuary environments tended to have birds with higher THg concentrations than those in freshwater environments. This difference could be due to several mechanisms, including differences in bioavailable methylmercury (such as differences in biogeochemical conditions, inorganic mercury availability, and methylmercury production; Ullrich et al., 2001), generally more complex food web structures and longer food-chain lengths in oceans and estuaries compared to smaller freshwater ecosystems (Post, 2002), or the ecology of bird species in these different habitats.

Blood-equivalent THg concentrations also differed among bird species ($F_{224,22076}$ =130.79, p < 0.0001; Figure 3; Figures S1–S7; Table S8). In particular, Forster's terns had the highest least squares mean blood-equivalent THg concentrations of any species with sample sizes 60 (Figure 3), which is the approximate sample size necessary to estimate a population's mean THg concentration with 10% accuracy (Ackerman et al., 2016b). Blood-equivalent geometric mean THg concentrations were 2.35 µg/g ww in Forster's terns (5th to 95th percentile: 0.87–6.39 µg/g ww; Table S8). For comparison, common loons in the west, another piscivore that is well studied throughout North America, had a blood-equivalent geometric mean THg concentration of 0.89 µg/g ww (5thto 95th percentile: 0.25–3.90 µg/g ww; Table S8). Some other species with notably high blood-equivalent geometric mean THg concentrations (2.08 µg/g ww), Caspian terns (1.58 µg/g ww), least terns (1.15 µg/g ww), black skimmers (0.90 µg/g ww), Clark's grebes (0.83 µg/g ww), and black-necked stilts (0.79 µg/g ww; Table S8).

Blood-equivalent THg concentrations of individual birds were above common toxicity benchmarks (Table 1) in many areas throughout western North America (Figure 1a). In particular, multiple individuals exhibited THg concentrations above $3.0 \ \mu g/g$ ww in San Francisco Bay, California; Central Valley, California; Carson River watershed, Nevada; Great Salt Lake, Utah; northeastern Washington; northeastern Montana; multiple sites along the Missouri River; southern Arizona; the Gulf Coast of Texas; Alaska's North Slope; and the Aleutian Archipelago. These individuals typically were from species belonging to upper trophic level guilds, such as piscivores and carnivores.

To examine spatial variation in mercury exposure of birds that accounted for differences in THg concentrations among species, the distribution of blood-equivalent THg concentrations in birds also were mapped using model-estimated least squares means within 100-km × 100-km grid cells across western North America. As expected, bird blood-equivalent THg concentrations differed among grid cells ($F_{431,26126}$ =20.67, p<0.0001; Figure 4a). Model-estimated mean THg concentrations were greatest in coastal California, western Nevada, and Alaska's North Slope (Figure 4a). Other apparent hotspots, such as those in other parts of Alaska, British Columbia, Hawaiian Islands, and the western contiguous United States, had high THg concentrations but low sample sizes (typically <15; Figure 4b) and high coefficients of variation (>25%; Figure 4c) making interpretation at these sites more difficult. The analysis was repeated separately for each guild with a sample size >5000 and similar results were generally found for the piscivore (Figure 5a), insectivore (Figure 5b), and omnivore guilds (Figure 5c). THg concentrations were compared among guilds when

they overlapped in the same grid cell. The strength of the correlations between guild-specific least squares mean blood-equivalent THg concentrations within grid cells varied among guilds, although the relationships were always positive (Pearson correlation s; omnivore vs insectivore: n=79 grid cells, r=0.47, p<0.0001; omnivore vs piscivore: n=56 grid cells, r=0.24, p=0.08; piscivore vs insectivore: n=69 grid cells, r=0.16, p=0.19).

3.2. Factors influencing bird mercury: literature review

Bird blood-equivalent least squares mean THg concentrations differed among foraging guilds ($F_{7,167,1}$ =16.01, p<0.0001; Figure 2a), habitat types ($F_{7,211}$ =2.86, p=0.01; Figure 2b), and ecoregions ($F_{14,283,3}$ =2.08, p=0.01; Figure 6). Carnivores (0.37±0.15 µg/g ww) and piscivores (0.31±0.09 µg/g ww) exhibited the greatest blood-equivalent least squares mean THg concentrations, whereas herbivores (0.01±0.01 µg/g ww) exhibited the lowest blood-equivalent least squares mean THg concentrations. As observed in the raw dataset, bird blood-equivalent least squares mean THg concentrations were highest in salt marsh (0.35±0.28 µg/g ww) and ocean (0.23±0.08 µg/g ww) habitats and lowest in terrestrial-ground habitats (0.04±0.01 µg/g ww). Among ecoregions, bird blood-equivalent least squares mean THg concentrations were greatest in tropical dry forests (0.22±0.13 µg/g ww) and tundra habitats (0.22±0.06 µg/g ww) and lowest in temperate Sierras (0.06±0.03 µg/g ww) and southern semi-arid highlands (0.04±0.02 µg/g ww), but pair-wise comparisons suggested few statistically significant differences among ecoregions (Figure 6).

Similar to the raw dataset, bird blood-equivalent mean THg concentrations differed among species ($F_{177,1427}$ =10.61, p<0.0001; Figures S8–S14) and grid cells ($F_{312,1195}$ =4.17, p<0.0001; Figure 7a). Model-estimated mean bird THg concentrations based on the literature data also were highest in central and coastal California, western Nevada, Alaska's North Slope, and the Aleutian Islands (Figure 7a). Additional hotspots were present throughout the west, although several of these additional sites had low sample sizes (typically <15; Figure 7b) and high coefficients of variation (>25%; Figure 7c). To directly compare the raw data (432 grid cells) to the literature data (313 grid cells), model-estimated mean bird THg concentrations within the 165 grid cells (100 km²) that contained both raw data and literature-review data were correlated. Least squares mean blood-equivalent THg concentrations were positively correlated between the two separate datasets, although the strength of the correlation was moderate (Pearson correlation; r=0.34; p<0.0001).

3.3. Hotspots of bird mercury contamination in western North America

From the raw and literature-review data analyses, hotspots were identified in western North America for mercury contamination in birds. Several of these identified hotspots were common to both the raw and literature-review datasets, including the western Aleutian Islands, Alaska's North Slope, Great Basin (especially western Nevada), and San Francisco Bay and Central Valley of California (Figures 4 and 7). To facilitate visualization of avian mercury exposure risk across western North America, a comprehensive map (Figure 8) was produced by combining the maps developed from the raw data and the literature-review data. When a grid cell contained THg concentration estimates from both analyses, priority was given to the estimate derived from the raw data and excluded the literature review-derived estimate for that grid cell. All grid cells that contained least squares mean blood-equivalent

THg concentrations that were above the 80th percentile of the entire dataset were considered to be potential hotspots for bird mercury contamination. Using this approach, 101 grid cells were identified that can be considered to be hotspots for avian mercury contamination in western North America (red grid cells in Figure 8). These hotspots included locations in the Aleutian Islands; the North Slope of Alaska; east-central Alaska; southeastern Alaska; northern Nunavut, Canada; Puget Sound, Washington; Great Basin (especially northern Idaho, and western and northern Nevada); San Francisco Bay and Central Valley, California; southern Arizona; the Gulf Coast of Texas; and the Hawaiian Islands (Figure 8).

Among the grid cell hotspots identified from the combination of the raw and literaturereview datasets, many were characterized by low sample sizes (<15 samples; n=1 grid cell), high coefficients of variation (>25%; n=7 grid cells), or both (n=71 grid cells). Thus, additional sampling in these locations would help to determine if they are hotspots for bird mercury contamination. On the other hand, 22 of the identified hotspots were well sampled (>15 samples) and had relatively low coefficients of variation (<25%). These identified hotspots (red grid cells with bolded black borders in Figure 8) included the North Slope of Alaska; the western Aleutian Islands; Puget Sound; southwestern Idaho; western Wyoming; northern Montana; North Dakota and South Dakota along the Missouri River; central Arizona; the Gulf Coast of Texas; western Nevada ; and San Francisco Bay, California. Similar hotspots of mercury contamination were observed at some sites for freshwater fishes, especially in western and northern Nevada and central Arizona (Eagles-Smith et al., submitted to this issue). Avian mercury hotspots on the North Slope of Alaska may reflect recent trends in increased mercury exposure observed in piscivorous birds in the Arctic (Evers et al., 2014; Rigét et al., 2011), which are thought to be related to atmospheric deposition (Blum et al., 2013; Sunderland et al., 2009) and warmer Arctic temperatures associated with climate change potentially releasing inorganic mercury within snowpack, permafrost, and sea ice, and enhancing methylmercury production (AMAP, 2002; Brooks et al., 2006). In the Aleutian Islands, several studies have demonstrated high THg concentrations in birds with concentrations sometimes increasing westward across the island chain (Anthony et al., 2007; Ricca et al., 2008). In Washington's Puget Sound, surf scoters exhibited THg concentrations similar to those of surf scoters in San Francisco Bay, California (Henny et al., 1991; Ohlendorf et al., 1987) and mercury concentrations of both surf scoters and western grebes increased as they over-wintered in Puget Sound (Henny et al., 1991, 1990). The hotspot in the Gulf Coast of Texas included Lavaca Bay, a designated mercury superfund site. Finally, San Francisco Bay estuary, California and western Nevada, have a long history of mercury contamination due to the legacy of mining (Conaway et al., 2008; Singer et al., 2013) and have widespread mercury contamination of biota (Ackerman et al., 2008, 2007; Eagles-Smith and Ackerman, 2014; Eagles-Smith et al., 2009a; Henny et al., 2007, 2002). San Francisco Bay, California; western Nevada ; and other Great Basin areas are of particular concern for methylmercury exposure to birds in western North America, and would benefit from inclusion in continental contaminant monitoring programs (Mason et al., 2005).

3.4. Literature review of mercury toxicity to birds and translation of toxicity benchmarks into a common blood-equivalent tissue

The literature was reviewed, the published toxicity benchmarks for birds were summarized, and toxicity benchmarks for different tissues were integrated into a common bloodequivalent THg concentration (Table 1). This approach provides the ability to integrate toxicity risk across avian tissues and life-stages into a single toxicity benchmark based on bird blood. Effects occurred across a range of blood-equivalent THg concentrations, with many documented effects in the range of 1.0 to 3.0 μ g/g ww and more severe effects occurring over $3.0 \,\mu g/g$ ww (Table 1). The lowest documented effects in birds occurred at a blood-equivalent THg concentration of 0.2 µg/g ww (Table 1). In general, health, physiology, behavior, and reproduction tended to be affected by methylmercury at lower blood-equivalent THg concentrations (1.0 μ g/g ww), substantial impairment to health and reproduction occurred at moderate blood-equivalent THg concentrations (2.0 μ g/g ww), more severe impairment to health and reproduction occurred at higher blood-equivalent THg concentrations (3.0 µg/g ww), and often complete reproductive failure occurred at extremely high blood-equivalent THg concentrations (4.0 µg/g ww; Table 1). THg concentrations in blood over 4.0 µg/g ww in bird blood resulted in a variety of severe physiological and reproductive effects, including adult mortality at blood-equivalent THg concentrations over $8.5 \,\mu\text{g/g}$ ww (Table 1).

At approximately $1.0 \,\mu g/g$ ww in bird blood, effects of methylmercury exposure included altered bird breeding behaviors (Frederick and Jayasena, 2010; Tartu et al., 2015); reduced breeding success of south polar skuas during the subsequent breeding season (Goutte et al., 2014); reduced egg hatchability (LC_{50} : lethal concentration where 50% mortality occurs) of highly-sensitive birds (Heinz et al., 2009b); an estimated 12% reduction in common loon productivity (Burgess and Meyer, 2008); reduced egg hatchability (LC 50) in thick-billed murres (Braune et al., 2012); the onset of demethylation of methymercury in the liver of Forster's terns, Caspian terns, American avocets, and black-necked stilts (Eagles-Smith et al., 2009b); changes to enzymes associated with glutathione metabolism and antioxidant activity in ruddy ducks (Hoffman et al., 1998); and impaired behavior of common loons (Depew et al., 2012). A bird blood-equivalent THg concentration of $1.0 \,\mu g/g$ ww also is very close to the derived toxicity benchmark for impaired bird reproduction using egg and liver tissue in the review by Shore et al. (2011). At approximately 2.0 μ g/g ww in bird blood, effects of methylmercury exposure included impaired reproduction in captive dosed mallards (Heinz, 1979); reduced egg hatchability (LC_{50}) of moderately-sensitive birds (Heinz et al., 2009b); reduced breeding success of brown skuas during the subsequent breeding season (Goutte et al., 2014); an estimated 23% reduction in common loon productivity (Burgess and Meyer, 2008); reduced egg hatchability (LC_{50}) in Arctic terns (Braune et al., 2012); and impaired productivity of common loons (Depew et al., 2012). At approximately 3.0 µg/g ww in bird blood, effects of methylmercury exposure included impaired productivity (Barr, 1986), reproductive failure (Depew et al., 2012; Evers et al., 2008), and a 35% reduction in the productivity of common loons (Burgess and Meyer, 2008); decreased immune competence in tree swallows (Hawley et al., 2009); and decreased egg hatchability in ringnecked pheasants (Fimreite, 1971). Finally, at approximately 4.0 µg/g ww in bird blood, effects of methylmercury exposure became widespread among most bird species and

included reduced egg hatchability (LC_{50}) of birds that are less-sensitive to methylmercury toxicity (Heinz et al., 2009b); reduced egg hatchability (LC_{50}) in common loons (Kenow et al., 2011); increased incidence of same-sex pairs (Frederick and Jayasena, 2010); and an estimated 50% reduction in common loon productivity (Burgess and Meyer, 2008).

Because sensitivity to methylmercury toxicity can differ widely among species (Heinz et al., 2009b), it is difficult to select a single toxicity benchmark that can be applied across species, such as for the 273 species included in this paper (Table S1). However, some general principles can be derived from the synthesis of published toxicity studies that can be used to guide the interpretation of bird methylmercury concentrations (Table 1). In general, birds with blood THg concentrations $<0.2 \ \mu g/g$ ww are below any known effect levels and can be considered to have background levels of methylmercury exposure. Birds with blood THg concentrations between 0.2–1.0 μ g/g ww can be considered to have lower risk, 1.0–3.0 μ g/g ww have moderate risk, $3.0-4.0 \ \mu g/g$ ww have higher risk, and >4.0 $\mu g/g$ ww have severe risk to methylmercury toxicity. Overall, 66% of individual birds exceeded a blood-equivalent THg concentration of 0.2 μ g/g ww (above background levels), 28% exceeded 1.0 μ g/g ww (moderate risk and above), 8% exceeded 3.0 µg/g ww (high risk and above), and 4% exceeded 4.0 μ g/g ww (severe risk; Table S9). Because numerous effects to health and reproduction occur in many bird species at blood THg concentrations near 3.0 µg/g ww (Table 1), that is a useful methylmercury toxicity benchmark for the potential for more severe impairment to bird health and reproduction. Species with>5% of individuals exceeding THg concentrations of 3.0 µg/g ww in blood included horned grebe (100%), black-footed albatross (44%), Forster's tern (33%), pigeon guillemot (30%), willet (25%), northern fulmar (23%), northern shoveler (19%), black skimmer (13%), Clark's grebe (11%), clapper rail (11%), American white pelican (11%), Caspian tern (10%), peregrine falcon (9%), least tern (9%), common loon (8%), double-crested cormorant (8%), blacknecked stilt (8%), Wilson's phalarope (8%), snowy plover (7%), and ruddy turnstone (7%; Table S9). Songbirds, in particular, may be more sensitive to methylmercury toxicity (Heinz et al., 2009b), and substantial impairment may occur at blood THg concentrations of only $1.0 \,\mu g/g$ ww (Table 1). The percentage of individual songbirds exceeding $1.0 \,\mu g/g$ ww included western kingbird (40%), bank swallow (20%), American robin (10%), yellowbreasted chat (7%), ash-throated flycatcher (4%), willow flycatcher (4%), tree swallow (3%), house wren (2%), rusty blackbird (2%), white-crowned sparrow (2%), and barn swallow (1%; Table S9). Table S9 can be used to examine additional species at a range of blood-equivalent THg concentrations from 0.2 to 4.0 µg/g ww. Figure 9 shows the proportion of individual birds exceeding various toxicity benchmarks only for those species with 60 samples. Often, there can be as much variability in THg concentrations among individuals of the same species as among species due to the substantially large influences of local site and habitat-specific effects on methylmercury production and bioaccumulation (Eagles-Smith et al., 2009a); therefore comparisons among species (Figures S2–S14) should be viewed as approximations of relative methylmercury exposure at this large scale of study.

4. Suggestions for mercury monitoring programs

To compile mercury contamination data in birds throughout western North America, many different datasets derived from seven different tissues (egg, albumen, whole blood, muscle,

liver, kidney, and feathers) were used. It was necessary to make several assumptions and to use general equations to translate these seven tissues into a common matrix - bloodequivalent THg concentrations – for comparisons among studies and species. These generalities introduced uncertainty into the resulting estimates of blood-equivalent THg concentrations, especially for tissues like adult feathers. These results suggest that future mercury monitoring efforts would benefit from sampling tissues that are most-easily translated into a tissue that has a well-developed toxicity benchmark and that is directly relevant to bird reproduction (Table 2). These high-priority sampling tissues include adult blood, eggs, and chick down feathers (in contrast to low-priority adult feathers). Bird THg concentrations in whole blood are highly correlated to THg and methylmercury concentrations in internal tissues that require more invasive sampling procedures (Eagles-Smith et al., 2008). Additionally, the THg concentration in a female's blood is highly correlated to THg concentrations in her eggs (Ackerman et al., 2016a), providing THg concentrations in blood with a strong link to the numerous toxicity benchmarks that have been developed for egg hatchability. Eggs are a high-priority sampling tissue because they are relatively easy to sample, and relate directly to many toxicity benchmarks, including impaired reproduction. Egg THg concentrations need to be reported on a fresh wet weight basis (Ackerman et al., 2013; Stickel et al., 1973), and therefore it is necessary to collect additional egg morphometric data (such as egg length, width, and weight) for proper adjustments to the measured egg THg concentrations. Down feathers also can be a useful tissue, because THg concentrations in down feathers represent in ovo exposure and can be translated into equivalent THg concentrations in whole eggs (Ackerman and Eagles-Smith, 2009). Besides chick down feathers, sampling juvenile birds for contaminant monitoring purposes is not advised, because THg concentrations in internal tissues (including blood) change rapidly as chicks age due to mass dilution and mercury transfer into growing feathers (Ackerman et al., 2011; Kenow et al., 2007) and, therefore, are difficult to interpret.

Tissues which have a moderate-priority for assessing bird contamination include egg albumen, that can be non-lethally sampled and translated into whole-egg THg concentrations (Ackerman and Eagles-Smith, 2009; Stebbins et al., 2009); and muscle, liver, kidney, and brain, which are highly correlated to other internal tissues, including whole blood (Eagles-Smith et al., 2008; Scheuhammer et al., 2008), but require more invasive sampling procedures. Additionally, unlike in blood, eggs, muscle, and feathers, most of the THg in the liver and kidney often is not in the methylmercury form due to the ability of birds to demethylate methylmercury within the liver, especially at high THg concentrations (Eagles-Smith et al., 2009b; Henny et al., 2002; Scheuhammer et al., 2008). Therefore, chemical determination of methylmercury, in addition to THg, may be necessary when using liver and kidney tissues. Finally, although many mercury monitoring programs use them, feathers have low-priority as a preferred tissue for sampling. Feather THg concentrations are highly variable within an individual bird (Bond and Diamond, 2008; Braune and Gaskin, 1987; Cristol et al., 2012; Furness et al., 1986), and are relatively poorly correlated with THg concentrations in internal tissues (Eagles-Smith et al., 2008; Evers et al., 1998) that are more likely to indicate risk of current methylmercury toxicity. Furthermore, THg concentrations in feathers represent THg concentrations in blood at the time of feather growth, which is a combination of the bird's body burden of mercury, via redistribution of mercury among

internal tissues during molt, and recent mercury acquired through diet (Braune and Gaskin, 1987; Furness et al., 1986; Thompson et al., 1998). Not only is the timing of feather molt often unknown, but molt may represent a time when internal mercury concentrations are rapidly changing due to mercury transfer to feathers (Ackerman et al., 2011; Condon and Cristol, 2009) and the often-associated nutritional stress. There are certainly exceptions where adult feathers may be useful for mercury monitoring, including (1) for non-migratory bird species with extremely small home ranges (or other ecology) which make THg concentrations in feathers highly correlated to those in internal tissues (Ackerman et al., 2012), (2) when more invasive sampling methods need to be avoided (such as endangered species), or (3) when using museum specimens to examine long-term temporal trends, because no other tissue is available (Bond et al., 2015; Monteiro and Furness, 1997).

In addition to selecting the most useful bird tissues, reasonable efforts to ensure adequate sample sizes are acquired are important for properly characterizing methylmercury risk to birds. Few studies have been published on this topic, but Ackerman et al. (2016b) demonstrated that to estimate a population's mean THg concentration using eggs would typically require >60 samples to be within 10% of the population's actual mean THg concentration. Similar sample sizes would be necessary for other bird populations when variance in THg concentrations is comparable to any of the three species in that study. Sampling fewer individuals will result in an estimate that has lower accuracy, but sampling 15–30 individuals will normally provide an estimate within 20% of the population's actual mean THg concentration (Ackerman et al., 2016b).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Blood-equivalent total mercury (THg) concentrations in birds across western North America using original data (*n*=27,629 individual samples). All individual data points are shown, with lower THg concentrations as larger symbols in the background and higher THg concentrations as smaller symbols in the foreground.



Figure 2.

Mean blood-equivalent total mercury (THg) concentrations in birds across western North America based on data derived from a literature review (n=1,712 means, representing n=19,998 individual samples). All mean data points are shown, with lower mean THg concentrations as larger symbols in the background and higher mean THg concentrations as smaller symbols in the foreground.



Figure 3.

Least squares (LS) mean \pm standard error blood-equivalent total mercury (THg) concentrations in birds among (**A**) foraging guilds and (**B**) habitats in western North America using original data at the individual level (black-filled bars; *n*=27,629 individual samples) and mean data derived from a literature review (hatched bars; *n*=1,712 means, representing *n*=19,998 individual samples). LS mean blood-equivalent THg concentrations were estimated separately for each dataset from models with foraging guild, habitat, and ecoregion as fixed effects, and grid cell, year, and species as random effects. Different letters

next to bars denote significant (p < 0.05) differences between means for the raw dataset (capital letters) and literature-review dataset (lower case letters).



Figure 4.

Least squares (LS) mean \pm standard error blood-equivalent total mercury (THg) concentrations among bird species in western North America using original data at the individual level. Only species with sample sizes 60 are displayed; see Figures S2–S7 for a complete listing of species by taxanomic order. LS mean blood-equivalent THg concentrations were estimated from a model with species as a fixed effect, and grid cell and year as random effects.





Figure 5.

Blood-equivalent total mercury (THg) concentrations in birds across western North America using raw data (n=27,629 individual samples). Each grid cell is 100 km × 100 km. (**A**) The large map on the opposite page displays grid cells by their percentile of least squares (LS) mean THg concentration relative to the entire dataset, such that 20% of all grid cells are represented by each color. LS mean THg concentrations were estimated from a model with grid cell as a fixed effect, and species and year as random effects. (**B**) Displays the sample size in each grid cell. (**C**) Displays the coefficient of variation (as a percentage) for the model-estimated LS mean THg concentration in each grid cell. The three maps can be used

in combination to evaluate the confidence in the estimated blood-equivalent THg concentration in individual grid cells. The darker graduations indicate (**B**) smaller sample sizes and (**C**) greater coefficients of variation which denote lower confidence in the model-estimated LS mean THg concentrations in those grid cells.



Figure 6.

Bird blood-equivalent total mercury (THg) concentrations in (**A**) piscivores (n=10,243 individual samples), (**B**) insectivores (n=8,464 individual samples), and (**C**) omnivores (n=6,685 individual samples) across western North America using raw data. Each grid cell is 100 km × 100 km. Maps display grid cells by their percentile of least squares (LS) mean THg concentration relative to the entire dataset, such that 20% of all grid cells in each foraging guild are represented by each color. LS mean THg concentrations in each foraging guild were estimated from a model with grid cell as a fixed effect, and species and year as random effects.



Figure 7.

Least squares (LS) mean \pm standard error blood-equivalent total mercury (THg) concentrations in birds among ecoregions in western North America using data derived from a literature review (*n*=1,712 means, representing *n*=19,998 individual samples). LS mean blood-equivalent THg concentrations were estimated from a model with foraging guild, habitat, and ecoregion as fixed effects, and grid cell, year, and species as random effects. Different lowercase letters next to bars denote significant (*p*<0.05) differences between means. Literature-derived bird THg concentrations were available for 15 of the possible 17 ecoregions in western North America.





Figure 8.

Blood-equivalent total mercury (THg) concentrations in birds across western North America based on data derived from a literature review (n=1,712 means, representing n=19,998 individual samples). Each grid cell is 100 km × 100 km. (**A**) The large map on the opposite page displays grid cells by their percentile of least squares (LS) mean THg concentration relative to the entire dataset, such that 20% of all grid cells are represented by each color. LS mean THg concentrations were estimated from a model with grid cell as a fixed effect, and species and year as random effects. (**B**) Displays the effective sample size in each grid cell. (**C**) Displays the coefficient of variation (as a percentage) for the model-estimated LS mean

THg concentration in each grid cell. The three maps can be used in combination to evaluate the confidence in the estimated blood-equivalent THg concentration in individual grid cells. The darker graduations indicate (**B**) smaller sample sizes and (**C**) greater coefficients of variation which denote lower confidence in the model-estimated LS mean THg concentrations in those grid cells.



Figure 9.

Blood-equivalent total mercury (THg) concentrations in birds across western North America using raw data (grid cells not hatched: n=27,629 individual samples) and mean data derived from a literature review (hatched grid cells: n=1,712 means, representing n=19,998 individual samples). Each grid cell is 100 km × 100 km. The map displays grid cells by their percentile of least squares (LS) mean THg concentration relative to the entire dataset, such that 20% of grid cells are represented by each color for each dataset. However, when grid cells had an estimated THg concentration using both the raw and literature-review datasets, priority was given to the raw data and the literature-derived estimate for that grid cell was excluded. LS mean THg concentrations were estimated separately for each dataset from models with grid cell as a fixed effect, and species and year as random effects. Red grid cells that are outlined in black indicate hotspots that were well sampled (>15 samples) and had relatively low coefficients of variation (<25%).



Figure 10.

Percentage of individual birds sampled in western North America that are at risk to methylmercury contamination based on blood-equivalent total mercury concentrations using raw data. Only species with 60 samples are included; see Table S9 for all species. Risk categories are: $<0.2 \ \mu g/g \ ww$ (blue; below any known effect levels), $0.2 \ to <1.0 \ \mu g/g \ ww$ (yellow; low risk), $1.0 \ to <3.0 \ \mu g/g \ ww$ (orange; moderate risk), $3.0 \ to <4.0 \ \mu g/g \ ww$ (red; high risk), and $4.0 \ \mu g/g \ ww$ (dark red; severe risk). Brackets on the right indicate groups of species where some individuals have blood-equivalent total mercury concentrations over the specified toxicity benchmark.

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Table 1

Summary of toxicity benchmarks for the effects of methylmercury exposure on birds. Toxicity benchmarks were translated from the original tissue from table is sorted from the lowest to the highest blood-equivalent total mercury concentration where a toxic effect of methylmercury on birds was observed. concentration; LC₅₀: lethal concentration where 50% mortality occurs; na = no equation was needed to translate into blood total mercury concentration. which they were derived into blood-equivalent units using correlational models of total mercury concentrations between blood and various tissues. The reliably translate chick total mercury concentrations into equivalent total mercury concentrations in adult blood. Acronyms are blood = whole blood; Effects on juvenile birds were excluded due to the temporal complexity of methylmercury concentrations in chicks as they age, and the inability to RBCs = red blood cells; ww = wet weight; dw = dry weight; fww = fresh wet weight; THg = total mercury concentration; MeHg = methylmercury

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		Blood-	Ō	iginal tissue TF	le			
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Impairment category	Hg toxicity effect	(MM)	Tissue	Benchmark	Units	Bird species	Study ^d	Blood-equivalent equation ^{0,c}
Health and physiology	Oxidative stress response: negative relationship with thiobarbituric acid activity (below this concentration) d	0.2	Liver	1.60	µg∕g dw	Lesser Scaup	Custer et al. (2000)	_
Health and physiology	Altered gene expression in females (below this concentration) d	0.3	RBCs	1.20	wb g/gµ	Double-crested Cormorant	Gibson et al. (2014)	7
Reproduction	Median for males that raised only 1 of 2 chicks; no males above this threshold successfully raised 2 chicks	0.3	RBCs	1.20	µg/g dw	Black-legged Kittiwake	Tartu et al. (2015b)	6
Reproduction	Decreased egg hatchability (mean of eggs from dosed females)	0.3	Egg	0.15	ww g/gµ	Ring-necked Pheasant	Spann et al. (1972) ^{<i>a</i>}	ω
Reproduction	Median for birds that skipped breeding (higher than birds that bred); altered hormones	0.4	RBCs	2.00	wb g/gµ	Black-legged Kittiwake	Tartu et al. (2013)	0
Behavioral	Increased egg neglect for males (lower concentrations had no observed egg neglect)	0.4	RBCs	2.00	wb g/gµ	Snow Petrel	Tartu et al. (2015a)	7
Reproduction	Egg hatchability: LC ₅₀ of egg- injected birds ranked as high sensitivity to MeHg	0.5	Egg	0.25	ww g/gµ	Multiple	Heinz et al. (2009a) ^a	ε
Reproduction	10% reduction in probability of nest success	0.7	Blood	0.70	ww g/gµ	Carolina Wren	Jackson et al. (2011)	na
Reproduction	13% decrease in productive nests; altered courtship behaviors (mean of dosed	0.7	Blood	0.73	ww g/gµ	White Ibis	Frederick and Jayasena (2010) ^a	na

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Impairment category	Hg toxicity effect	equivalent THg (μg/g ww)	Tissue	Benchmark	Units	Bird species	Study^{a}	Blood-equivalent equation ^{b,c}
Reproduction	Probability of breeding successfully the subsequent year drops below 50%	0.8	RBCs	3.90	µg∕g dw	South Polar Skua	Goutte et al. (2014)	2
Reproduction	Proposed indicative concentration for impaired reproduction (review)	0.8	Liver	2.00	ww g/gµ	Multiple	Shore et al. (2011)	-
Reproduction	10% reduction in max. productivity	0.0	Blood	06.0	ww g/gµ	Common Loon	Burgess and Meyer (2008)	na
Health and physiology	Negative relationship with cortisol (below this concentration) d	1.0	Blood	1.00	ww g/gµ	Tree Swallow	Franceschini et al. (2009)	na
Reproduction	Decreased egg hatchability	1.1	Egg	0.50	ww g∕gµ	Ring-necked Pheasant	Fimreite (1971)	3
Health and physiology	MeHg demethylation threshold in liver	1.2	Liver	8.51	wb g/gµ	Forster's Tern, Caspian Tern, American Avocet, Black-necked Stilt	Eagles-Smith et al. (2009b)	-
Reproduction	20% reduction in probability of nest success	1.2	Blood	1.20	ww g/gµ	Carolina Wren	Jackson et al. (2011)	na
Reproduction	Egg hatchability: LC ₅₀ of egg- injected and maternally derived MeHg	1.2	В B B B B B B B B B B B B B B B B B B B	0.56	ww g/gµ	Thick-billed Murre	Braune et al. $(2012)^{a}$	σ
Health and physiology	Glutathione metabolism and antioxidant activity (effect on associated enzymes below this concentration) d	1.2	Liver	9.00	µg/g dw	Ruddy Duck	Hoffman et al. (1998)	-
Reproduction	Decrease in productivity	1.3	Egg	3.00	wb g/gµ	Merlin	Newton and Haas (1988)	ω
Reproduction	Proposed indicative concentration for impaired reproduction (review)	1.3	Egg Bg	0.60	ww g/gµ	Multiple	Shore et al. (2011)	ς,
Behavioral	Impaired behavior (review)	1.4	Diet (fish)	0.10	ww g/gµ	Common Loon	Depew et al. (2012)	4
Health and physiology	Negative relationship with body condition (below this concentration) ^d	1.6	Blood	1.56	ww g/gµ	Clapper Rail	Ackerman et al. (2012)	па
Reproduction	Decreased egg hatchability (mean of contaminated site)	1.6	Egg	2.86	wb g/gµ	House Wren	Custer et al. (2007)	ю
Reproduction	15% decrease in productive nests; altered courtship behaviors (mean of dosed birds)	1.6	Blood	1.60	ww g/gµ	White Ibis	Frederick and Jayasena (2010) ^a	na

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		Blood-	Or	iginal tissue TH	Ig			
Impairment category	Hg toxicity effect	equivalent THg (µg/g ww)	Tissue	Benchmark	Units	Bird species	Studv ^a	Blood-equivalent equation b,c
Reproduction	30% reduction in probability of nest success	1.7	Blood	1.70	ww g/gµ	Carolina Wren	Jackson et al. (2011)	, un
Reproduction	Impaired reproduction	1.8	Egg	0.80	ww g/gµ	Mallard	Heinz (1979) ^{<i>a</i>}	3
Reproduction	23% reduction in max. productivity	2.0	Blood	2.00	ww g/gµ	Common Loon	Burgess and Meyer (2008)	па
Health and physiology	Proposed concentration for adverse effects in waterbirds (review)	2.0	Liver	5.00	ww g/gµ	Multiple	Zillioux et al. (1993)	Ч
Reproduction	Impaired productivity (review)	2.1	Diet (fish)	0.18	ww g/gµ	Common Loon	Depew et al. (2012)	4
Reproduction	Probability of successfully raising 2 chicks the subsequent year drops below 50%	2.1	RBCs	10.00	µg/g dw	Brown Skua	Goutte et al. (2014)	2
Reproduction	40% reduction in probability of nest success	2.1	Blood	2.10	ww g/gµ	Carolina Wren	Jackson et al. (2011)	па
Reproduction	General impaired hatchability and embryonic mortality (review)	2.3	Egg	1.00	µg/g fww	Multiple	Scheuhammer et al. (2007)	ε
Reproduction	Egg hatchability: LC ₅₀ of egg- injected birds ranked as moderate sensitivity to MeHg	2.3	Egg	1.00	ww g/gµ	Multiple	Heinz et al. (2009a) ^a	ñ
Reproduction	50% reduction in probability of nest success	2.5	Blood	2.50	ww g/gµ	Carolina Wren	Jackson et al. (2011)	па
Reproduction	Egg hatchability: LC ₅₀ of egg- injected and maternally derived MeHg	2.5	Egg	1.10	ww g/gµ	Arctic Tern	Braune et al. (2012) ^a	ε
Reproduction	10% probability of embryo being malpositioned in egg	2.7	Egg	1.20	µg/g fww	Forster's Tern	Herring et al. (2010)	ю
Reproduction	Impaired productivity	2.8	Diet (fish)	0.30	ww g/gµ	Common Loon	Barr (1986)	4
Health and physiology	Decreased immunocompetence (mean of contaminated site)	2.9	Blood	2.85	ww g/gµ	Tree Swallow	Hawley et al. (2009)	па
Reproduction	35% reduction in max. productivity	3.0	Blood	3.00	ww g/gц	Common Loon	Burgess and Meyer (2008)	па
Reproduction	Reproductive failure	3.0	Blood	3.00	ww g/gµ	Common Loon	Evers et al. (2008)	na
Reproduction	Decreased hatching and fiedging success when ambient temps. increased (mean of contaminated site)	3.0	Blood	3.03	ww g/gu	Tree Swallow	Hallinger and Cristol (2011)	па

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		Blood-	Ori	ginal tissue TF	Ig			
Impairment category	Hg toxicity effect	equivalent THg (µg/g ww)	Tissue	Benchmark	Units	Bird species	Study ^a	Blood-equivalent equation ^{b,c}
Health and physiology	Suggested threshold above which demethylation occurs in a dose dependent relationship	3.2	Liver	8.00	µg∕g ww	Black-crowned Night- heron, Snowy Egret, Double-crested Cormorant	Henny et al. (2002)	-
Reproduction	20% probability of embryo being malpositioned in egg	3.2	Egg	1.40	µg/g fww	Forster's Tem	Herring et al. (2010)	з
Reproduction	Failed productivity (review)	3.4	Diet (fish)	0.40	ww g∕gµ	Common Loon	Depew et al. (2012)	4
Reproduction	Severe impaired productivity	3.4	Diet (fish)	0.40	ww g∕gµ	Common Loon	Barr (1986)	4
Reproduction	Decreased egg hatchability	3.5	Egg	1.50	ww g∕gµ	Ring-necked Pheasant	Fimreite (1971)	3
Reproduction	Decreased productivity for first time breeding females (in 1 of 2 years of study; mean of contaminated site)	3.6	Blood	3.56	ww g/gц	Tree Swallow	Brasso and Cristol (2008)	па
Reproduction	30% probability of embryo being malpositioned in egg	3.6	Egg	1.55	µg/g fww	Forster's Tern	Herring et al. (2010)	б
Reproduction	14% decrease in productive nests; altered courtship behaviors; higher proportion of same sex nest pairs (mean of dosed birds)	4.0	Blood	3.95	ww g/gµ	White Ibis	Frederick and Jayasena (2010) ^a	Па
Reproduction	40% probability of embryo being malpositioned in egg	4.0	Egg	1.69	µg/g fww	Forster's Tern	Herring et al. (2010)	з
Reproduction	46% reduction in max. productivity	4.0	Blood	4.00	ww g/gµ	Common Loon	Burgess and Meyer (2008)	па
Reproduction	16% reduction in reproductive success	4.0	Blood	4.00	ww g/gµ	Zebra Finch	Varian-Ramos et al. (2014) ^a	па
Reproduction	Egg hatchability: LC ₅₀ of egg- injected and maternally derived MeHg	4.2	Egg	1.78	ww g/gµ	Common Loon	Kenow et al. $(2011)^{a}$	m
Reproduction	Egg hatchability: LC ₅₀ of egg- injected birds ranked as low sensitivity to MeHg	4.2	Egg	1.79	ww g/gµ	Multiple	Heinz et al. (2009a) ^{<i>a</i>}	ς,
Reproduction	50% probability of embryo being malpositioned in egg	4.3	Egg	1.82	µg/g fww	Forster's Tern	Herring et al. (2010)	б
Reproduction	50% reduction in max. productivity	4.3	Blood	4.30	ww g/gµ	Common Loon	Burgess and Meyer (2008)	па
Reproduction	Decreased egg hatchability (mean of contaminated site)	4.3	Egg	7.34	wb g/gµ	Tree Swallow	Custer et al. (2007)	ω
Health and physiology	Glutathione metabolism and antioxidant activity (effect on	4.6	Liver	35.00	µg∕g dw	Surf Scoter	Hoffman et al. (1998)	-

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		Blood-	Ō	iginal tissue TH	50			
Impairment category	Hg toxicity effect	equivalent THg (µg/g ww)	Tissue	Benchmark	Units	Bird species	Study ^a	Blood-equivalent equation b,c
	associated enzymes below this concentration) d							
Reproduction	24% decline in young fledged per pair (over this concentration)	4.8	Egg	2.00	ww g/gu	American Kestrel	Albers et al. $(2007)^{a}$	ω
Health and physiology	Impaired macrophage phagocytosis (below this concentration) d	6.4	Blood	6.40	ww g/gµ	Black-footed Albatross	Finkelstein et al. (2007)	na
Reproduction	Decreased offspring survival (mean of dosed birds)	6.6	Muscle	4.50	ww g/gµ	Black Duck	Finley and Stendell (1978) ^a	ŝ
Reproduction	31% reduction in reproductive success, greater number of days to renesting (mean of dosed birds)	8.0	Blood	8.00	ww g/gц	Zebra Finch	Varian-Ramos et al. (2014) ^a	па
Health and physiology	Glutathione metabolism and antioxidant activity (effect on associated enzymes below this concentration) ^d	8.5	Liver	66.00	µg∕g dw	Greater Scaup	Hoffinan et al. (1998)	1
Mortality	Proposed indicative concentration for death (review)	8.5	Liver	22.00	ww g/gu	Multiple	Shore et al. (2011)	Ι
Health and physiology	Effects on some bioindicators of oxidative stress (below this concentration) d	8.8	Liver	69.00	µg/g dw	Forster's Tern, Caspian Tern	Hoffman et al. (2011)	Ι
Reproduction	Decreased offspring survival (mean of dosed birds)	0.6	Liver	23.10	ww g/gµ	Black Duck	Finley and Stendell (1978) ^a	1
Reproduction	Reproductive impairment (mean from lake with decreased reproduction)	9.1	Egg	3.65	ww g/gµ	Common Tern	Fimreite (1974)	ω
Health and physiology	Decreased energy expenditure for flight takeoff; altered molt sequence (mean of dosed birds)	9.8	Blood	9.80	ww g/gµ	European Starling	Carlson et al. (2014) ^a	па
Mortality	Proposed concentration for mercury toxicity (review)	11.5	Liver	30.00	ww g/gµ	Multiple	Thompson (1996)	1
Reproduction	Lethality to embryo (mean of eggs from dosed females)	13.0	Egg	5.10	ww g/gµ	Black Duck	Finley and Stendell (1978) ²⁴ , from Shore et al. (2011)	ς
Behavioral	Mass loss and altered foraging behavior in response to	13.9	Blood	13.93	ww g/gµ	Zebra Finch	Kobiela et al. (2015) ^a	na

		Blood-	10	iginal tissue TF	lg B			
Impairment category	Hg toxicity effect	equivalent THg (µg/g ww)	Tissue	Benchmark	Units	Bird species	Study ^a	Blood-equivalent equation b,c
	simulated predator (mean of dosed birds)							
Behavioral	Visible neurotoxicity; impaired movement (mean of dosed birds)	16.4	Liver	43.00	ww g/gµ	Zebra Finch	Scheuhammer (1988) ^{<i>a</i>}	<i>a</i>
Reproduction	42% reduction in reproductive success, greater number of days to renesting (mean of dosed birds)	17.0	Blood	17.00	ww g/gu	Zebra Finch	Varian-Ramos et al. (2014) ^a	па
Mortality	Death; swelling of axons; loss of myelin (below this concentration) d	18.1	Muscle	11.40	g/gµ	Red-tailed Hawk	Fimreite and Karstad (1971) ^a	5 e
Mortality	Death (mean concentration for dead birds: review)	23.7	Liver	63.00	ww g/gц	Multiple	Shore et al. (2011)	1 <i>c</i>
Health and physiology	Decreased enzymes associated with oxidative stress (mean of dosed birds)	24.4	Liver	65.00	ww g/gµ	Mallard	Hoffman and Heinz (1998) ^a	<i>o</i> [
Mortality	Death (mean of dosed birds that died)	27.3	Liver	73.00	ww g/gµ	Zebra Finch	Scheuhammer (1988) ^{<i>a</i>}	1 <i>c</i>
Reproduction	50% reduction in reproductive success, greater number of days to renesting (mean of dosed birds)	31.0	Blood	31.00	ww g/gµ	Zebra Finch	Varian-Ramos et al. (2014) ^a	na
Health and physiology	Acute inflammatory response; physiological stress (mean of dosed birds)	41.7	Blood	41.71	ww g/gµ	American Kestrel	Fallacara et al. (2011) ^a	na
Mortality	Visible neurotoxicity; some death (mean of dosed birds)	45.0	Blood	45.00	ww g/gµ	American Kestrel	Bennett et al. (2009) ^a	na
Health and physiology	Effects on brain neurotransmitters (below this concentration) d	48.2	Liver	397.00	ур dw	Bald Eagle	Scheuhammer et al. (2008)	<u>0</u>
Mortality	Death (mean of dosed birds that died)	51.4	Muscle	30.00	ww g/gц	Grackle	Finley et al. (1979) ^{<i>a</i>}	5 e
Mortality	Death (mean of dosed birds that died)	54.2	Muscle	31.50	ww g/gµ	Cowbird	Finley et al. (1979) ^{<i>a</i>}	5 <i>e</i>
Health and physiology	Decreased ability to mount a stress response (below this concentration) d	57.0	Blood	57.00	ww g/gµ	Zebra Finch	Moore et al. (2014) ^a	па

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		Blood-	Or	iginal tissue TH	.60			
Impairment category	Hg toxicity effect	equivalent THg (µg/g ww)	Tissue	Benchmark	Units	Bird species	Study ^d	Blood-equivalent equation b,c
Health and physiology	Effects on brain neurotransmitters (below this concentration) d	65.2	Liver	542.00	wb g/gµ	Common Loon	Scheuhammer et al. (2008)	1 <i>e</i>
Mortality	Death (mean of dosed birds that died)	71.4	Muscle	40.70	ww g/gµ	Starling	Finley et al. (1979) ^a	5 e
Mortality	Death (mean of dosed birds that died)	94.0	Blood	94.00	ww g/gµ	American Kestrel	Bennett et al. $(2009)^{a}$	na
Mortality	Death (mean of dosed birds that died)	103.0	Muscle	57.10	µg∕g ww	Redwing Blackbird	Finley et al. (1979) ^{<i>a</i>}	5e
a								

Indicates a captive feeding study with dosed birds.

 $b_{\rm Equations}$ used to translate toxicity benchmark to bird blood-equivalent units:

(eq 1) ln
$$\left(Blood\ THg\frac{\mu a}{g}ww\right) = 0.970 \times \ln\left(Bird\ Liver\ THg\frac{\mu a}{g}dw\right) - 1.929$$
 (R² = 0.88; Eagles-Smith et al., 2008)

(eq 2) Results for THg concentrations in red blood cells were reported as µg/g dw, without any estimate of percent moisture available. Therefore, a percent moisture of 79% (see Eagles-Smith et al., 2008) was assumed to convert µg/g dw to µg/g ww.

(eq 3) ln
$$\left(Female Bird Blood THg_{\frac{g}{g}ww}\right) = 1.0734 \times \ln\left(Egg THg_{\frac{\mu g}{g}fww}\right) + 0.8149 (\mathbb{R}^2 = 0.95; \text{Ackerman et al., 2016a})$$

(eq 4) ln $\left(Female Bird Blood THg_{\frac{g}{g}ww}\right) = 0.6182 \times \ln\left(Preg Fish THg_{\frac{\mu g}{g}fww}\right) + 1.788 (\text{Ackerman et al., 2015})$
(eq 5) ln $\left(Blood THg_{\frac{g}{g}ww}\right) = 1.080 \times \ln\left(Bird Muscle THg_{\frac{g}{g}dw}\right) - 1.024 (\mathbb{R}^2 = 0.90; \text{Eagles-Smith et al., 2008})$

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^CMoisture content from the study was used if reported. If it was not reported, a moisture content of 67% in liver, 75% in eggs, 70% in muscle, and 79% in blood was used (Eagles-Smith et al., 2008). If wet weight vs dry weight was not reported, wet weight (muscle) was assumed.

d For correlative studies with a relationship between THg concentration and an effect, the highest observed THg concentration was reported and stated that the relationship was observed "below this concentration."

e^rTHg concentrations in these captive studies had highly-dosed birds with liver or muscle THg concentrations outside of the range of data used to generate the equations to translate tissue THg concentrations to blood-equivalent units, and blood-equivalent THg concentrations should be interpreted with caution.

Suggested	tissues	s for sampling bird me	ercury contaminati	ion.			
Priority	Age	Tissue	Mercury Analysis	Most THg in MeHg form?	Units	Represents	Reference
High	Adult	Blood	THg	Yes	wet weight or dry weight	Hg in adult and egg (if a breeding female)	Henny et al. (2002); Evers et al. (2003); Rimmer et al. (2005); Eagles-Smith et al. (2008); Brasso et al. (2010); Heinz et al. (2010); Kenow et al. (2015); Ou et al. (2015); Ackerman et al. (2016a)
High	Egg	Eggs	THg	Yes	fresh wet weight	Hg in egg and adult; direct link to reproduction	Ackerman et al. (2013); Ackerman et al. (2016a)
High	Chick	Feathers (down)	THg	Yes	dry weight	Hg in egg; highly correlated	Ackerman and Eagles-Smith (2009); Kenow et al. (2011)
Moderate	Egg	Egg albumen	THg	Yes	wet weight	Hg in whole egg; direct link to reproduction	Kennamer et al. (2005); Bond and Diamond (2009); Stebbins et al. (2009)
Moderate	Adult	Muscle	THg	Yes	dry weight	Hg in adult and egg (if a breeding female)	Finley and Stendell (1978); Scheuhammer et al. (1998); Eagles-Smith et al. (2008); Ackerman et al. (2016a)
Moderate	Adult	Liver	MeHg	No	dry weight	Hg in adult and egg (if a breeding female)	Finley and Stendell (1978); Henny et al. (2002); Eagles-Smith et al. (2008); Eagles-Smith et al. (2009b); Ackerman et al. (2016a)
Moderate	Adult	Kidney	MeHg	No	dry weight	Hg in adult and egg (if a breeding female)	Finley and Stendell (1978); Henny et al. (2002); Eagles-Smith et al. (2008); Ackerman et al. (2016a)
Moderate	Adult	Brain	MeHg	No	dry weight	Hg in adult	Finley and Stendell (1978); Scheuhammer et al. (2008)
Low	Adult	Feathers (fully-grown)	THg	Yes	dry weight	Poor correlation with Hg in internal tissues and eggs for most birds; exceptions are for species with limited movements	Thompson and Furness (1989); Brasso and Cristol (2008); Eagles-Smith et al. (2008); Jackson et al. (2011); Ackerman et al. (2012); Ackerman et al. (2016a)
Low	Egg	Egg yolk	THg	Yes	wet weight	Hg in whole egg; moderate correlation	Kennamer et al. (2005); Bond and Diamond (2009)
Low	Egg	Egg shell	THg	Unknown	dry weight	Hg in whole egg; moderate correlation	Kennamer et al. (2005)
Low	Chick	Blood	THg	Yes	wet weight or dry weight	Hg changes rapidly with chick age	Kenow et al. (2007); Ackerman et al. (2011)
Extra Low	Chick	Feathers (fully-grown)	THg	Yes	dry weight	Very poor correlation with Hg in internal tissues	Ackerman et al. (2009)
Extra Low	Adult	Feathers (primary flight feathers)	THg	Yes	dry weight	Very poor correlation with Hg in internal tissues; large variability among feathers and along length of feather	Furness et al. (1986); Braune and Gaskin (1987); Braune et al. (1987); Dauwe et al. (2003)

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Table 2