

Mercury exposure and neurochemical impacts in bald eagles across several Great Lakes states

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Abstract In this study, we assessed mercury (Hg) exposure in several tissues (brain, liver, and breast and primary feathers) in bald eagles (*Haliaeetus leucocephalus*) collected from across five Great Lakes states (Iowa, Michigan, Minnesota, Ohio, and Wisconsin) between 2002–2010, and assessed relationships between brain Hg and neurochemical receptors (NMDA and GABA_A) and enzymes (glutamine synthetase (GS) and glutamic acid decarboxylase (GAD)). Brain total Hg (THg) levels (dry weight basis) averaged 2.80 µg/g (range: 0.2–34.01), and levels were highest in Michigan birds. THg levels in liver ($r_p = 0.805$) and breast feathers ($r_p = 0.611$) significantly correlated with those in brain. Brain Hg was not associated with binding to the GABA_A receptor. Brain THg and inorganic

Hg (IHg) were significantly positively correlated with GS activity (THg $r_p = 0.190$; IHg $r_p = 0.188$) and negatively correlated with NMDA receptor levels (THg $r_p = -0.245$; IHg $r_p = -0.282$), and IHg was negatively correlated with GAD activity ($r_s = -0.196$). We also report upon Hg demethylation and relationships between Hg and Se in brain and liver. These results suggest that bald eagles in the Great Lakes region are exposed to Hg at levels capable of causing subclinical neurological damage, and that when tissue burdens are related to proposed avian thresholds approximately 14–27% of eagles studied here may be at risk.

Keywords Avian toxicology · Neurochemistry · Neurotoxicology · Biomarker · Monitoring · Methylmercury

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Introduction

Bald eagles (*Haliaeetus leucocephalus*), like other long-lived piscivorous birds, bioaccumulate mercury (Hg). Numerous studies on bald eagles across North America have documented appreciable Hg levels in eggs (Weimeyer et al. 1993), feathers (Bowerman et al. 1994; Wood et al. 1996; Weech et al. 2006; Bechard et al. 2007), blood (Wood et al. 1996; Weech et al. 2006), liver (Wood et al. 1996; Weech et al. 2003; Scheuhammer et al. 2008), and brain (Scheuhammer et al. 2008). Though Hg exposure in bald eagles is fairly well understood, much less is known about adverse health effects that may be associated with such exposures.

It is well established that Hg is neurotoxic. Methylmercury (MeHg) in fish readily crosses the blood–brain barrier and, due to Hg's affinity for protein thiols, may

target and disrupt receptors, enzymes, and other cellular components of the brain (Clarkson and Magos 2006). Pathologically, exposure to high Hg levels is associated with demyelination, neuronal degeneration, and inflammation in birds (Spalding et al. 2000). Perhaps due to underlying changes to the brain, Hg exposure has been linked to impaired motor skills and cognitive behaviors in species including common loons (Evers et al. 2008; Kenow et al. 2010), American kestrels (Bennett et al. 2009), and great egrets (Spalding et al. 2000). Such neurobehavioral changes potentially affect survival and reproduction. Mercury is associated with decreased reproductive success in the laboratory (e.g., American kestrels; Albers et al. 2007) and field (e.g., common loons; Burgess and Meyer 2008; Evers et al. 2008). Because impacts on survival and reproduction may affect both individual and population health, it is important to develop markers that may help recognize early, subclinical effects of Hg on the brain.

Alterations in neurochemical receptors and enzymes relate to brain function and indicate neurological damage before structural and functional toxicity are apparent (Manzo et al. 1996). Recently, several studies in fish-eating wildlife have identified associations between Hg and neurochemical biomarkers involved in various neurotransmitter systems. Species studied include mink (Basu et al. 2007b), river otters (Basu et al. 2007a), polar bears (Basu et al. 2009a), common loons, and bald eagles (Scheuhammer et al. 2008). Comparable effects have been seen in vitro and in animals exposed to MeHg in laboratory studies (Basu et al. 2005, 2006, 2007b). Collectively, these studies demonstrate that Hg exposure may cause subtle neurological impacts on many species.

Receptors and enzymes that mediate glutamate and γ -aminobutyric acid (GABA), the primary excitatory and inhibitory neurotransmitters, are tightly regulated and disruption can result in excitotoxicity (Reis et al. 2009). Rodent studies show that Hg alters components of both pathways (Concas et al. 1983; Rajanna et al. 1997; Monnet-Tschudi et al. 1996), but these effects are not well documented in birds. Because glutamate and GABA play roles in learning and coordination (Scholes 1965; Gibbs et al. 2008), changes in components of these systems may disrupt ecologically important behaviors.

To date, much of the work on Hg in eagles has been limited to monitoring studies assessing levels in feathers or blood. A recent study demonstrated associations between brain Hg levels and neurochemical receptors (*N*-methyl-D-aspartic acid (NMDA), muscarinic cholinergic) in bald eagles from Canadian provinces (Scheuhammer et al. 2008), thus suggesting that Hg may be of neurological concern to this species. Although Hg is of concern to Great Lakes ecosystem health, levels in the brain and related neurological effects in eagles in the region are unknown.

The first goal of this study was to assess Hg exposure (total (THg), organic (OHg), and inorganic (IHg) Hg), through measurements in brain, liver, and feathers, in bald eagles collected from five U.S. states in the Great Lakes region (IA, OH, MI, MN, and WI) from 2002 to 2010. The second goal was to determine if brain Hg levels were associated with changes in neurochemical receptors (NMDA and GABA_A (benzodiazepine)) and enzymes (glutamic acid decarboxylase (GAD), glutamine synthetase (GS)) involved in glutamate and GABA neurotransmission, most of which have not been investigated in wildlife toxicology studies.

Methods

Animals

Carcasses ($n = 135$) were provided by Michigan Department of Natural Resources and Environment, Ohio Department of Natural Resources, Saving our Avian Resources (Iowa), Wisconsin Department of Natural Resources, The Raptor Center (University of Minnesota), and Minnesota Department of Natural Resources. Birds died of various causes in the field or during rehabilitation. Carcasses were collected between 2002 and 2010 and stored at -20°C until tissue (liver, whole brain, breast feather (whole), and primary feather (distal 2 cm)) collection. Samples from all age classes were studied. Of birds for which sex data were available, 35% were male and 65% were female. Extracted tissues were stored at -80°C until all analyses. Proper USFWS permits were obtained for this work.

Hg analysis

Total and organic Hg were analyzed using methods described previously (Nam et al. 2010). Prior to analysis, brain and liver tissues were dried at 60°C for 48 h and crushed into a fine powder. Feathers were washed three times in acetone then deionized water, and were minced using stainless steel scissors and dried at room temperature for 24 h. OHg was extracted from dried liver and brain using micro-scale organic extraction methods described elsewhere (Nam et al. 2010). THg and OHg were measured by thermal decomposition, amalgamation, and atomic absorption spectrophotometry (Basu et al. 2009a) in a Direct Mercury Analyzer (DMA-80, Milestone Inc., CT). Intermittent blanks, duplicate samples and standard reference materials (SRMs) (TORT-2, DOLT-3, and DOLT-4, National Research Council of Canada) were included to monitor accuracy and precision. For THg, average accuracy was 98% of certified values and average precision (% relative standard deviation)

was 5%. For OHg, average accuracy was 104% of certified values and average precision was 20%. Residue information is reported on a dry weight basis. Inorganic Hg was calculated by subtracting OHg from THg. Detection limits for THg ranged from 0.03 to 1.04 ng and detection limits for OHg ranged from 0.03 to 2.09 ng.

Se analysis

Selenium (Se) was analyzed in liver and brain from a subsample of 46 eagles. Selenium was detected using an Inductively Coupled Plasma Mass Spectrometer (ICPMS; Agilent 7500c, Agilent Technologies, Palo Alto, CA) equipped with a quadrupole analyzer and octopole collision/reaction cell pressurized with hydrogen or helium reaction gas. All samples were digested overnight in 70% nitric acid (as 2% nitric acid solution) in a single batch run. Sample uptake was 0.4 ml/min from a peristaltic pump with 1.2 l/min Ar carrier gas through a Babbington-style nebulizer into a Peltier-cooled double-pass spray-chamber at 2°C; 1.0 l/min auxiliary Ar and 12.0 l/min plasma gas Ar were added for a total of 14.2 l/min separated from nickel cones by a sampling depth of 8.5 mm. The ICPMS was tuned using a solution of 10 ppb of Li, Y, Ce, Tl, and Co (Agilent internal standard mix). Interference levels were reduced by optimizing plasma conditions to produce low oxide and doubly charged ions (formation ratio of <1.0%) and residual matrix interferences were removed using the collision/reaction processes in the Octopole Reaction System. Residue information is reported on a dry weight basis. The limit of detection, determined as the mean of blanks plus 3 times the standard deviation of the mean, was 0.024 ng/g. Accuracy and precision from SRMs (DOLT-4) was within 12% of expected values.

Neurochemical assays

Cellular homogenates, enzyme supernatants, and membranes were prepared according to modifications of a procedure described elsewhere (Basu et al. 2006). Brains were homogenized in 50 mM Tris buffer (50 mM Tris HCl, 50 mM Tris Base, pH 7.4). Supernatant was collected after centrifuging at $15000\times g$ for 15 min., and membranes were isolated by centrifuging at $48000\times g$ for 15 min., washing three times, and resuspending in Tris buffer. The Bradford assay was used to determine protein content. Prepared samples were stored at -80°C .

GS activity was measured in supernatant based on the assay described by Santoro et al. (2001) with changes described below. Hydroxylamine hydrochloride and imidazole concentrations were increased to 50 mM and 100 mM, and sodium arsenate was used in place of arsenic acid. Assays were performed on supernatant (400 $\mu\text{g/ml}$) in

1.7 ml microcentrifuge tubes and incubation was in a 37°C shaking heating block. Absorbance was read at 500 nm on a Nanodrop ND-1000 spectrophotometer (Thermo Scientific) and values were compared to a standard curve to determine glutamyl-g-hydroxamate concentration and specific activity. Samples were assayed in triplicate and a pooled control sample was used to monitor variability. Between day variability averaged 10% and within day variability averaged 7%.

GAD activity was measured in brain homogenate according to a modification of previously described methods (Basu et al. 2009b). The reaction mixture was added to a 75 mm glass tube containing 50 μl (100 μg) of homogenate. This tube was placed in a 100 mm glass tube and a Whatman GF/A filter soaked in 100 μl Scintigest (Fisher Scientific, USA) was placed between the tubes. The reaction was started with the addition of 10 mM glutamate containing 0.3 μCi l-[U- ^{14}C] glutamic acid (260 mCi/mmol; Perkin Elmer, USA) and the tube was sealed and incubated for 45 min in a 37°C shaking heating block. The reaction was terminated with injection of 0.5 ml of 0.25 M HCl and tubes were incubated for an additional 60 min. After soaking in OptiPhase Supermix Cocktail (Perkin Elmer) for 24 h, radioactivity on the filters was measured on a liquid scintillation counter (Wallac 1450 Microbeta Plus, Perkin Elmer). Samples were assayed in duplicate and a pooled control sample was used to monitor variability. Between-day variability averaged 17% and within day variability averaged 13%.

Receptor binding assays were performed using cellular membranes according to previously published methods (Basu et al. 2009a). For both receptors, protein (30 μg) was suspended in 100 μl buffer (NMDA: 50 mM Tris buffer (pH 7.4) containing 100 μM glycine and glutamate; GABA: 50 mM Tris buffer (pH 7.4)). Samples were incubated at room temperature with 5 nM [3H]-MK-801 (27.5 Ci/mmol) for 2 h. (NMDA) or on ice with 2 nM [3H]-flunitrazepam (81.4 Ci/mmol) for 30 min. (GABA). Nonspecific binding was determined through coincubation with 100 μM unlabeled MK-801 (NMDA) or 20 μM clonazepam (GABA). Samples were assayed in quadruplicate and variability, which averaged 15%, was monitored through internal, pooled controls. Binding is reported as fmol of radioisotope bound per mg of membrane protein (fmol/mg).

Statistical analyses

Statistical analyses were performed using PASW Statistics (V.17.0, Chicago, IL, USA) and Stata (V.11) (for quantile regression). A p -value of <0.05 was considered significant. Hg and Se data were log transformed prior to analyses to achieve a normal distribution, and were back transformed for reporting. THg levels were compared across states

using one-way ANOVA (Tukey's post hoc). Correlations (Pearson or Spearman) were used to determine relationships between Hg concentrations in different tissues and between Hg or Se concentrations and neurochemical endpoints. Simple linear regression was used to determine equations when appropriate. Quantile regression was performed to investigate Hg as a limiting factor for GAD activity (Cade et al. 1999). ANOVA and *t*-tests were used to compare neurochemical endpoints between age and gender categories.

Results

Brain Hg and Se

An overview of THg concentrations in brain is provided in Table 1. In birds for which sex information was available, concentrations did not differ significantly between sexes. Mean concentrations of brain THg increased with age group and differences were detected between groups (Table 1). Brain THg varied significantly among the states (Fig. 1). On average, 64.1% (range: 2.8–98.0%) of THg in the brain was organic. Generally, demethylation increased as THg in the brain increased.

Levels of Se in the brain averaged 3.24 ± 2.54 µg/g (range: 1.17–17.92). Brain Se was positively correlated with brain THg ($p < 0.001$, $r_p = 0.732$), OHg ($p = 0.003$, $r_p = 0.434$), and IHg ($p < 0.001$, $r_p = 0.685$). The mean molar ratio of THg:Se was 0.38 ± 0.26 (range: 0.06–1.24). The molar concentration of THg surpassed that of Se in the brain of only one bird. The molar ratio of THg:Se correlated positively with THg ($p < 0.001$, $r_s = 0.957$), OHg ($p < 0.001$, $r_s = 0.670$), and IHg ($p < 0.001$, $r_s = 0.810$).

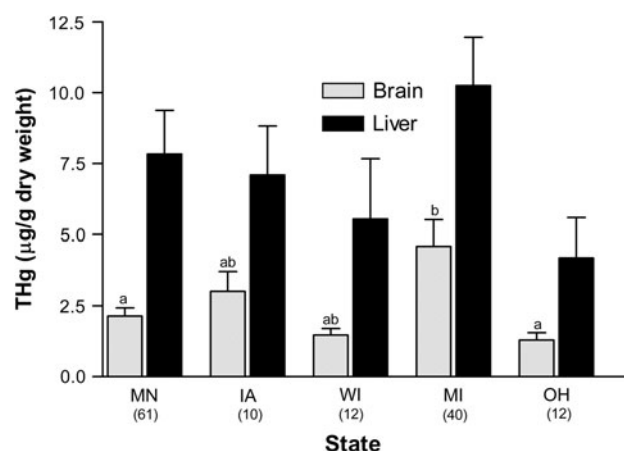


Fig. 1 Total mercury (THg) in brain and liver of wild bald eagles from Minnesota (MN), Iowa (IA), Wisconsin (WI), Michigan (MI), and Ohio (OH) (2002–2010). Sample sizes are indicated in parenthesis. Bars represent mean $\pm$ SE and letters denote significant differences (bars that do not share a letter are significantly different) between states ($p < 0.02$)

Liver Hg and Se

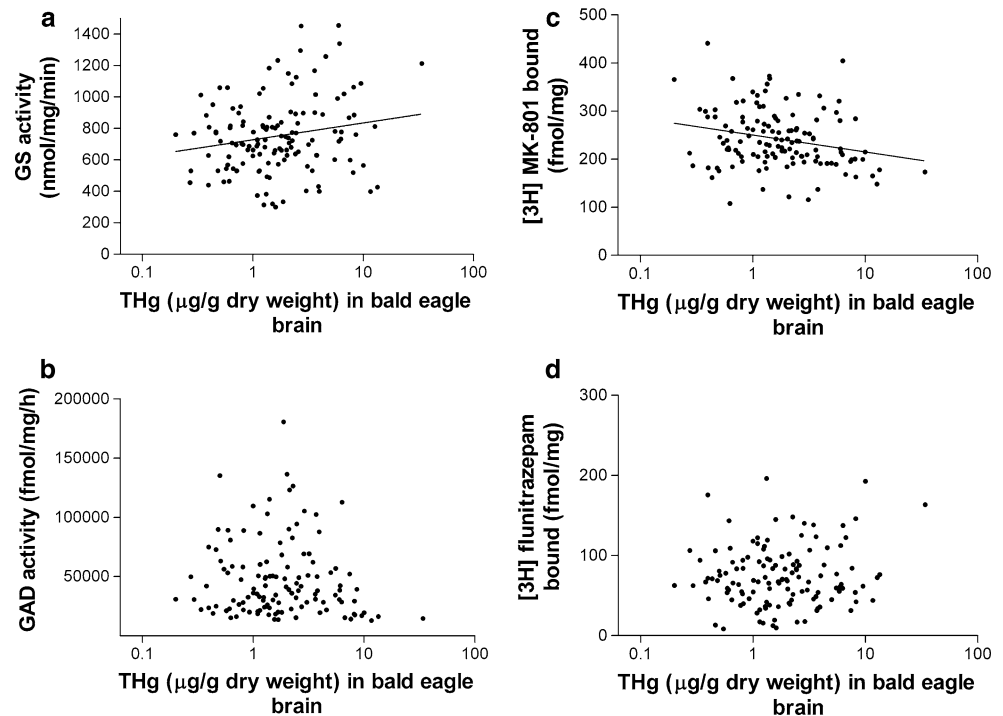
THg concentrations in the liver (Table 1) were higher than those in the brain. Concentrations did not differ significantly among states (Fig. 1). THg concentrations in liver were positively correlated with those in brain ($p < 0.001$, $r_p = 0.805$, $\log\text{BrainTHg} = -0.197 + 0.684 \log\text{LiverTHg}$). The average percent of THg in the organic form was 59.33% (range: 11–107%) and as in brain demethylation increased with THg concentrations. Se concentrations in liver averaged 6.07 ± 3.47 µg/g (range: 1.33–16.9). As in brain, Se was positively correlated with THg ($p < 0.001$, $r_p = 0.774$), OHg ($p = 0.003$, $r_p = 0.434$), and IHg ($p < 0.001$, $r_p = 0.685$) in liver.

Table 1 Mean concentrations ($\pm$ standard deviation) of total mercury (THg) (µg/g dry weight) and organic mercury (OHg, as a percentage of THg) in whole brain and liver from wild collected bald eagle carcasses collected between 2002–2010, stratified by sex and age class

	Brain		Liver	
	THg (µg/g)	Percent OHg	THg (µg/g)	Percent OHg
All eagles	2.80 ± 3.78 (135)	64.09 ± 25.14 (134)	7.97 ± 10.51 (135)	59.33 ± 22.64 (125)
Sex				
Male	4.40 ± 6.78 (24)	58.84 ± 29.13 (24)	11.40 ± 13.90 (24)	49.18 ± 22.90 (23)
Female	2.71 ± 2.65 (45)	56.03 ± 25.17 (45)	8.81 ± 11.64 (45)	55.69 ± 19.56 (45)
Unknown	2.28 ± 2.67 (66)	71.6 ± 21.43 (65)	6.15 ± 7.72 (66)	66.31 ± 22.92 (57)
Age class				
Nestling	0.52 ± 0.16 (8) ^a	80.94 ± 21.48 (8)	0.90 ± 0.33 (9) ^a	94.91 ± 11.25 (6)
Juvenile	1.03 ± 0.60 (11) ^{abc}	71.33 ± 20.85 (11)	2.42 ± 1.80 (11) ^a	74.46 ± 22.34 (10)
Subadult	2.67 ± 3.44 (20) ^{cd}	78.05 ± 11.96 (20)	8.78 ± 10.53 (20) ^b	63.74 ± 18.77 (20)
Adult	3.40 ± 4.52 (75) ^{de}	56.72 ± 27.35 (75)	9.46 ± 12.03 (75) ^b	53.64 ± 19.82 (73)
Unknown	2.59 ± 1.89 (21)	67.01 ± 19.76 (20)	7.97 ± 10.51 (20)	56.99 ± 26.72 (16)

Letters denote significant differences ($p < 0.01$) between groups. Samples sizes are indicated in parenthesis

Fig. 2 Associations between total mercury (THg) and **a** glutamine synthetase (GS) activity, ($r_p = 0.190$, $p = 0.028$, $n = 133$), **b** glutamic acid decarboxylase (GAD) activity ($r_s = -0.101$, $p = 0.269$, $n = 123$), **c** *N*-methyl-D-aspartic acid (NMDA) receptor levels ($r_p = -0.245$, $p = 0.005$, $n = 128$), and **d** γ -aminobutyric acid (GABA_A) benzodiazepine receptor levels ($r_p = 0.102$, $p = 0.252$, $n = 128$) in the brain of wild bald eagles collected between 2002–2010). Dashed lines represent best fit linear regression



Unlike the brain, in the liver there was less of an excess of Se over Hg and the molar ratio of THg:Se approached or exceeded 1 in several birds. The mean molar ratio of THg:Se in the liver was 0.50 ± 0.42 (range: 0.5–2.4). The ratio was positively correlated with liver THg levels ($p < 0.001$, $r_s = 0.912$).

Feather Hg

THg in breast feathers averaged 15.84 ± 13.49 $\mu\text{g/g}$ (range: 0.54–75.05; $n = 106$). THg in primary feathers averaged 15.31 ± 10.63 $\mu\text{g/g}$ (range: 1.73–15.31; $n = 50$). THg in breast feathers correlated with THg in primary feathers ($p < 0.001$, $r_s = 0.617$), liver ($p < 0.001$, $r_p = 0.610$), and brain ($p < 0.001$, $r_p = 0.611$, $\log\text{BrainTHg} = -0.357 + 0.630 \log\text{BreastFeatherTHg}$). Though THg in primary feathers increased with THg in brain and liver, these relationships were not statistically significant.

Neurochemical assays

To assess relationships between Hg and neurochemical biomarkers, receptor levels and enzyme activity were plotted against log transformed THg, OHg, and IHg. Brain Thg was associated with an increase in GS activity ($p = 0.028$, $r_p = 0.190$) (Fig. 2a). When OHg and IHg were examined separately, the relationship with GS was significant for IHg ($p = 0.030$, $r_p = 0.188$) but not OHg. GAD activity was not significantly associated with brain THg (Fig. 2b) or OHg, but was negatively associated with

IHg ($p = 0.03$, $r_s = -0.196$). The significance of a curve fit at the 85th percentile in a quantile regression ($p = 0.05$, $b_0 = 525.42$, $b_1 = -12.85$) suggests that THg may be a limiting factor for GAD activity. Based on this analysis, concentrations of 10 and 20 $\mu\text{g/g}$ brain THg would be associated with decreases in maximum GAD activity of 25% and 50% compared to unexposed birds. For OHg, the 90th quantile was significant ($p = 0.03$, $b_0 = 646.64$, $b_1 = -63.64$) but quantile regression did not predict that IHg was a limiting factor for GS activity. Binding to the NMDA receptor decreased in association with brain THg ($p = 0.005$, $r_p = -0.245$) (Fig. 2c) and IHg ($p = 0.001$, $r_p = -0.282$), but not OHg. Binding to the GABA_A (benzodiazepine) receptor was not associated with brain THg (Fig. 2d), OHg, or IHg. Associations between brain Se and neurochemical receptors and enzymes were also investigated. GS activity and NMDA and GABA receptor binding did not correlate significantly with brain Se or the brain Hg:Se ratio. GAD activity was negatively correlated with brain Se ($p = 0.005$, $r_s = -0.430$) but not the Hg:Se ratio. Neither age nor gender were significantly associated with any neurochemical parameter.

Discussion

The current study is only the second to investigate brain Hg levels in bald eagles, and we found that THg levels in these eagles from the US Great Lakes (0.2–34.01 $\mu\text{g/g}$) were comparable to those found in eagles from Canada

(0.3–23 µg/g; Scheuhammer et al. 2008). Although no threshold has been derived for Hg's effects on the avian brain, levels found in the current study have been associated with neurochemical change in loons, eagles (Scheuhammer et al. 2008), and several wild mammals (Basu et al. 2007a, 2009a). Our results also support those of Scheuhammer et al. (2008) showing demethylation occurs in the bald eagle brain, particularly in birds with higher concentrations of THg. This is of interest because the form of the metal affects its toxicity, sequestration, and elimination from the body (Clarkson and Magos 2006). Also as in the Scheuhammer et al. (2008) study, Hg was correlated with Se and the Hg:Se ratio. Accumulation of Se represents an important detoxification mechanism (Clarkson and Magos 2006), and that the Hg:Se ratio rarely exceeded 1 suggests that Se may offer some protection against Hg toxicity.

In our study, concentrations of THg in the liver averaged 7.97 µg/g and ranged from 0.47 to 61.61 µg/g. Bald eagles in British Columbia, Canada from 1987 to 1994 had a mean liver concentration of 11.8 µg/g and most birds fell in a range from 0.5 to 17.2 µg/g (Weech et al. 2003). Liver concentrations in adult bald eagles collected in Florida from 1987 to 1993 ranged from 2.17 to 42.07 µg/g (converted from dry weight; Wood et al. 1996). Eagles collected across Canada during the 1990s also had a similar range of 0.5 to 104 µg/g as well as similar relationships between THg and Se and the Hg:Se ratio (Scheuhammer et al. 2008). A conservative liver threshold for toxic effects in waterbirds is 16.7 µg/g (converted from wet weight assuming 70% moisture; Zillioux et al. 1993), which was exceeded by 14% of birds in our study. We measured on average 15 µg/g THg in breast and primary feathers. Mean feather concentrations in eagles in Idaho (Bechard et al. 2007) and three British Columbia reference sites (Weech et al. 2006) were 18.74 and 9.3 µg/g, 13 and 14 µg/g, respectively. Concentrations in primary feathers collected across the Great Lakes from 1985 to 1989, which ranged from 3.6 to 48 µg/g (Bowerman et al. 1994), were slightly higher than those found in our more recent study. Though we lack information regarding habitat differences that may explain disparities, this may suggest a decreasing trend in Hg contamination in eagles which is supported by findings that breast feather Hg levels declined 2.4% annually from 1991 to 2008 in eagles along Lake Superior (Dykstra et al. 2010). Scheuhammer (1991) suggested that levels above 20 µg/g in raptor feathers might be of concern. Evers et al. (2008) identified 40 µg/g in feathers as a threshold for adverse effects in loons. In our study 27% of eagles had levels over 20 µg/g, and 7% had levels over 40 µg/g in breast feathers. From proposed liver and feather thresholds, our regression equations relating these tissues to brain Hg predict a brain threshold for toxicity of approximately

4.5 µg/g, which is exceeded by 17% of eagles in this study. However, it must be noted that these thresholds do not consider dietary Se, which likely influences thresholds in individuals.

Although the brain is a key site for Hg toxicity, monitoring studies usually rely on levels in feathers and blood, which may be sampled non-lethally, to assess trends and health risks. Ideally, levels in these tissues would predict risks for neurotoxic effects. In this study we measured Hg in several tissues and evaluated liver, breast feathers, and primary feathers as predictors of levels in brain. As in previous eagle work (Scheuhammer et al. 2008), liver THg correlated highly with brain THg suggesting that levels in liver may give a fair indication of risk for neurotoxic effects. We found that breast feathers may be useful for predicting brain Hg concentrations, but caution should be used when extrapolating brain levels from breast feather levels as the correlation between brain and breast feather THg, though significant, was weaker than that with liver THg. Primary feather THg was not significantly correlated with brain THg and therefore may not be useful to predict brain levels. These findings are similar to those of Hopkins et al. (2008) demonstrating that, although body feathers better predict tissue levels than do flight feathers, feathers are not always indicative of the Hg body burden in osprey (Hopkins et al. 2008). Nonetheless, feather collection is an important non-lethal technique for long-term monitoring of Hg in live birds.

We also investigated spatial patterns in brain Hg concentrations and most notably found that birds in MI had higher levels than birds in other states. No other study has reported brain Hg levels in eagles from the Great Lakes, but a study of feathers sampled from 1985 to 1989 reported the lowest Hg levels in birds from Lake Erie and little variation in birds from the more western Great Lakes (Bowerman et al. 1994). Likewise, we found low brain Hg levels in birds from Ohio, which borders Lake Erie. However, we did find some brain Hg differences between birds in states bordering more western lakes. The discrepancy in spatial trends between the two studies may be due to temporal trends, as reported by Dykstra et al. (2010), habitat differences, or to the weak correlation between feather and brain Hg that we identified.

We examined relationships between Hg exposure and four neurochemical biomarkers, including three that were previously unstudied in wildlife. Consistent with a previous study on eagles (Scheuhammer et al. 2008), we observed a Hg-associated decrease in NMDA receptor levels. Hg toxicity is characterized by an increase in synaptic glutamate as reuptake by astrocytes is inhibited (Aschner 1996). Because extracellular glutamate is toxic (Albrecht and Matyja 1996), this decrease in NMDA receptors may be an adaptation to prevent excitotoxicity. When coupled with

several studies demonstrating a similar Hg-associated decrease in NMDA receptors (Basu et al. 2007b, 2009a; Scheuhammer et al. 2008), our results help support the notion that the NMDA receptor is a sensitive indicator of Hg's earliest neurological effects. We found no relationship between Hg and GABA_A receptor benzodiazepine receptors, which are known to increase with Hg exposure in rats (Concas et al. 1983). We observed a slight, though significant increase in GS activity with brain THg levels. While this enzyme has been inhibited by Hg in vitro (Monnet-Tschudi et al. 1996), one rat study found a Hg associated increase in vivo (Kung et al. 1989). These disparate results may be explained by in vitro work showing that extracellular glutamate, which is common in Hg toxicity, increases GS expression (Lehmann et al. 2009). With GAD, we found a significant association with IHg, but not with OHg or THg. Results of previous in vivo studies vary, with some showing no effect of Hg treatment on GAD activity (Tsuzuki 1981; Concas et al. 1983) and others showing a decrease in activity (O'Kusky and McGeer 1985; O'Kusky et al. 1988). However, these studies do not relate activity to brain levels of THg, OHg, or IHg. Both OHg and IHg inhibit GAD (Monnet-Tschudi et al. 1996; Basu et al. 2010) and IHg is more potent (Basu et al. 2010), which could explain our findings. Another interesting aspect to the relationship is that quantile regression suggests THg may be a limiting factor for GAD activity. This type of relationship with THg, which is represented by a wedge shaped plot, has been seen with hormone concentrations in minnows (Drevnick and Sandheinrich 2003), productivity in loons (Burgess and Meyer 2008) and neurochemical enzyme activity in mink (Basu et al. 2007a), and indicates that Hg may impose an upper limit on GAD activity. However, owing to the tremendous variability in enzyme activity further work is required to interpret the relationship between Hg and GAD.

In conclusion, this study demonstrates that many bald eagles in the Great Lakes region accumulate substantial levels of Hg and provides evidence that Hg is associated with neurochemical changes. Similar changes have been identified in mink (Basu et al. 2007b), otters (Basu et al. 2007a), polar bears (Basu et al. 2009a), loons, and eagles (Scheuhammer et al. 2008), but the relevance of these subclinical changes is unknown. Considering that Hg is known to affect learning, memory, and motor coordination (Evers et al. 2008; Bennett et al. 2009; Spalding et al. 2000; Kenow et al. 2010), which are in part regulated by glutamate and GABA (Scholes 1965; Gibbs et al. 2008), changes in these neurotransmitters may impact ecologically important behaviors in wildlife. Future work should focus on identifying a threshold for Hg's subclinical effects on the brain and clarifying the relationship between subclinical changes and relevant behavioral changes.

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