BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
PROPOSED NEW 35 ILL. ADM. CODE 225	)	R06-25
CONTROL OF EMISSIONS FROM	)	(Rulemaking – Air)
LARGE COMBUSTION SOURCES (MERCURY)	)	

NOTICE OF FILING

PLEASE TAKE NOTICE that the Environmental Law and Policy Center has filed the attached MICHAEL MURRAY REFERENCES IN SUPPORT OF TESTIMONY.

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DATED: August 8, 2006

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MICHAEL MURRAY REFERENCES IN SUPPORT OF TESTIMONY

The following documents are references in support of the testimony of Michael Murray which was filed in PCB R06-25 on July 24, 2006.

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Patterns and Interpretation of Mercury Exposure in Freshwater Avian Communities in Northeastern North America

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Abstract. A large data set of over 4,700 records of avian mercury (Hg) levels in northeastern North America was compiled and evaluated. As Hg emissions remain poorly regulated in the United States and Canada, atmospheric deposition patterns and associated ecological responses continue to elicit interest by landscape managers, conservation biologists, policy makers, and the general public. How avian Hg exposure is interpreted greatly influences decision-making practices. The geographic extent and size of this data set is valuable in understanding the factors that affect the exposure of Hg to birds. Featured are differences found among tissues, major aquatic habitats and geographic areas, between age class and gender, and among species. While Hg concentrations in egg and blood reflect short-term Hg exposure, Hg concentrations in liver and feather provide insight into long-term Hg exposure. Blood is a particularly important matrix for relating site-specific exposure to methylmercury (MeHg). The level of MeHg is generally 5–10x greater in adults compared to nestlings. Age also influences MeHg bioaccumulation, particularly for individuals where MeHg intake exceeds elimination. Gender is of interpretive concern when evaluating Hg exposure for species exhibiting sexual dimorphism and niche partitioning. Based on two indicator species, the belted kingfisher (*Ceryle alcyon*) and bald eagle (*Haliaeetus leucocephalus*), we found MeHg availability increased from marine, to estuarine and riverine systems, and was greatest in lake habitats. A large sample of >1,800 blood and egg Hg levels from the common loon (*Gavia immer*) facilitated a suitable comparison of geographic differences. Although some clusters of highly elevated Hg exposure (i.e., blood levels > 3.0 μg/g, ww and egg levels > 1.3 μg/g, ww) were associated with hydrological and biogeochemical factors known to increase MeHg production and availability, others were not. Geographic areas without a relationship between Hg exposure and biogeochemical processes were associated with emission or waterborne point sources. Differences in Hg exposure among species are primarily correlated with trophic position and availability of MeHg. Although piscivorous species were repeatedly

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shown to have some of the highest MeHg levels of the 38 species analyzed, insectivorous birds in both aquatic and terrestrial habitats (such as montane areas) were also found with elevated MeHg levels. A better understanding of the factors confounding interpretation of Hg exposure provides an effective basis for choice of indicator species and tissues according to 12 selected scenarios. This and the national need for spatiotemporal monitoring of MeHg availability require careful consideration of indicator species choice. Only then will local, regional, continental, and even global monitoring efforts be effective.

Keywords: bird; loon; methylmercury; monitoring; indicator species

Introduction

The ecological impact from atmospheric deposition of mercury (Hg) has emerged as a major global environmental issue. Global concerns stem from the broad geographic extent of contamination, the increasing global signal of Hg deposition, and, until recently, a general lack of regulations to control many uses and the disposal of Hg (United Nations Environment Programme, 2003). In North America, decades of increasing Hg deposition appear to have reversed in some areas (Engstrom and Swain, 1997; Schuster et al., 2002; Fevold et al., 2003), including the Northeast (Kamman and Engstrom, 2002), but the need to identify and monitor ecological changes remains a high priority (Mason et al., 2005). Federal, state and/or provincial regulation of atmospheric mercury emissions in the United States and Canada is in place for some industrial sectors (i.e., municipal and medical waste incineration), but is currently lacking for others (i.e., coal-fired electrical generators and mining). Not all environmental Hg is related to atmospheric deposition. Many past and even current inputs of waterborne Hg sources occur throughout North America and the Northeast. These are related to past improper waste disposal of Hg at weapons facilities (Halbrook et al., 1999), chlor-alkali plants (Fimreite, 1974; Gardner et al., 1978; Barr, 1986; Adair et al., 2003), mercury, gold, and silver mines (Elbert and Anderson, 1998; Henny et al., 2002; Seiler et al., 2004; Weech et al., 2004) and governmental storage facilities (Moore et al., 1999) as well as current inputs from wastewater treatment plants (Glass et al., 1990).

The U.S. Environmental Protection Agency (USEPA) investigated the ecological impacts of Hg based on key wildlife species as a basis for potential regulatory actions (USEPA 1997). An outgrowth of this effort was the development of a generic wildlife criterion value for bird and mammal species (Nichols and Bradbury, 1999). Since the USEPA Report to Congress (USEPA1997), scientific investigations on the biogeochemical process of methylmercury production and availability have dramatically improved our basic knowledge (Morel et al., 1998; Lucotte et al., 1999; Wiener et al., 2003). A better understanding of the mechanisms of Hg transfer and fate has improved the ability to predict methylmercury (MeHg) production and availability (USEPA 2002), particularly in freshwater habitats of northeastern North America (Evers and Clair, 2005). This has resulted in a greater insight into now identifying specific geographic areas and biota at greatest risk to Hg exposure and effects.

Birds are at particularly high risk to Hg toxicity because many species are at high trophic levels (e.g., susceptible to biomagnification), are long-lived (e.g., susceptible to bioaccumulation), are vulnerable to neurological and reproductive impacts from elevated Hg levels, and are frequently subjected to multiple anthropogenic stressors.

Using birds as bioindicators of MeHg availability

The use of piscivorous birds as bioindicators of MeHg availability and risk in freshwater systems is common (e.g., Fimreite, 1974; Barr, 1986; Scheuhammer, 1987; Wolfe et al., 1998; Rumbold et al., 2001; Henny et al., 2002; Evers et al., 2003), although insectivorous birds are increasingly being used as well (Wolfe and Norman, 1998; Gerrard and St. Louis, 2001; Adair et al., 2003). Historically, Hg exposure was primarily determined by killing birds and was therefore based on organs analysis (Thompson, 1996). Although collection of viable eggs continues to be a relevant lethal method widely used (Braune et al., 2001), non-lethal sampling efforts based on blood (Bowerman et al., 2002;

Evers et al., 1998; Fevold et al., 2003), feathers (Burger, 1993), and abandoned eggs (Scheuhammer et al., 2001; Evers et al., 2003) are increasingly a more frequently used approach. Since Hg concentrations in different avian tissues reflect different temporal scales of past Hg exposure, care must be taken in considering Hg pharmacokinetics when selecting the best avian tissue to match specific biomonitoring objectives.

This paper represents a three-year effort through the U.S. Department of Agriculture's Northeastern States Research Cooperative (NSRC) to comprehensively compile and synthesize bird Hg data across northeastern North America. The paper's purpose is to describe this large data set and use the information to identify and assess the importance of factors that affect exposure and bioaccumulation of Hg.

Methods

Source data sets

We targeted the collection of Hg data in birds from aquatic freshwater systems in New England, New York, and eastern Canada (eastern Ontario to the Canadian Maritimes) (Fig. 1). The Great Lakes and Lake Champlain were not included within our data set. Only blood Hg data for belted kingfishers and bald eagles were gathered from saltwater systems; these data were used to demonstrate differences among major aquatic habitats (Fig. 2). The majority of data (>90%) were provided by BioDiversity Research Institute, Canadian Wildlife Service, U.S. Environmental Protection Agency, and the U.S. Fish and Wildlife Service.

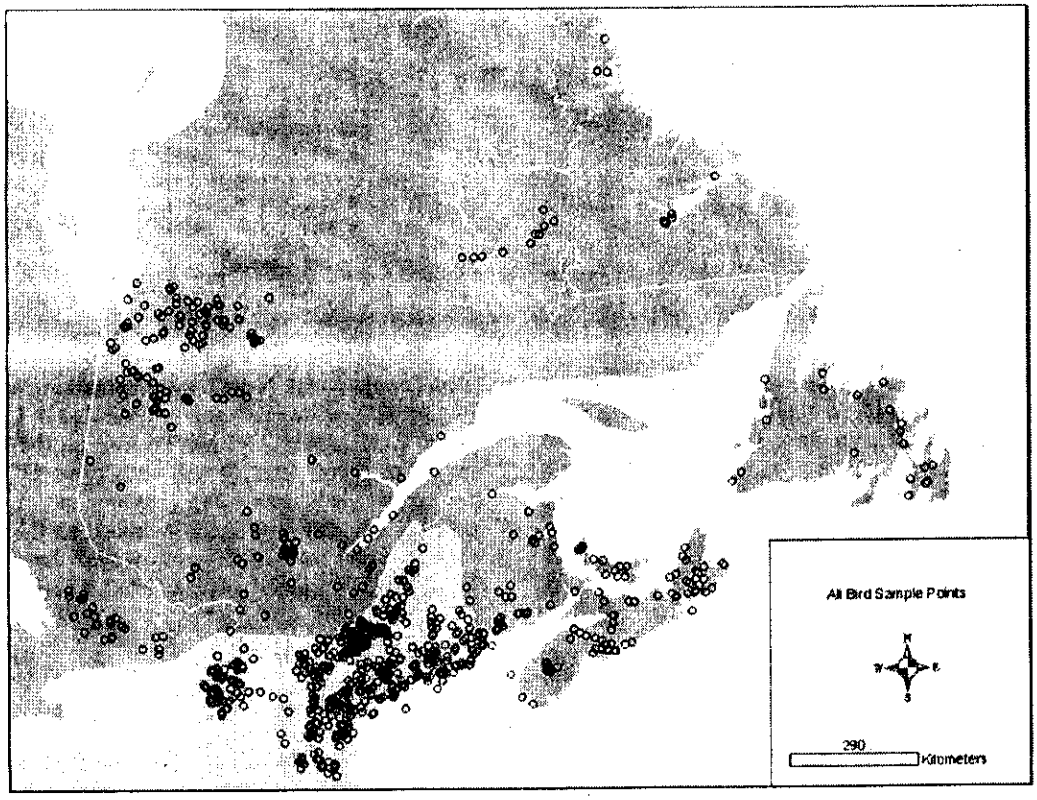

Figure 1. Distribution of Hg sampling effort for all bird species, 1969–2003.

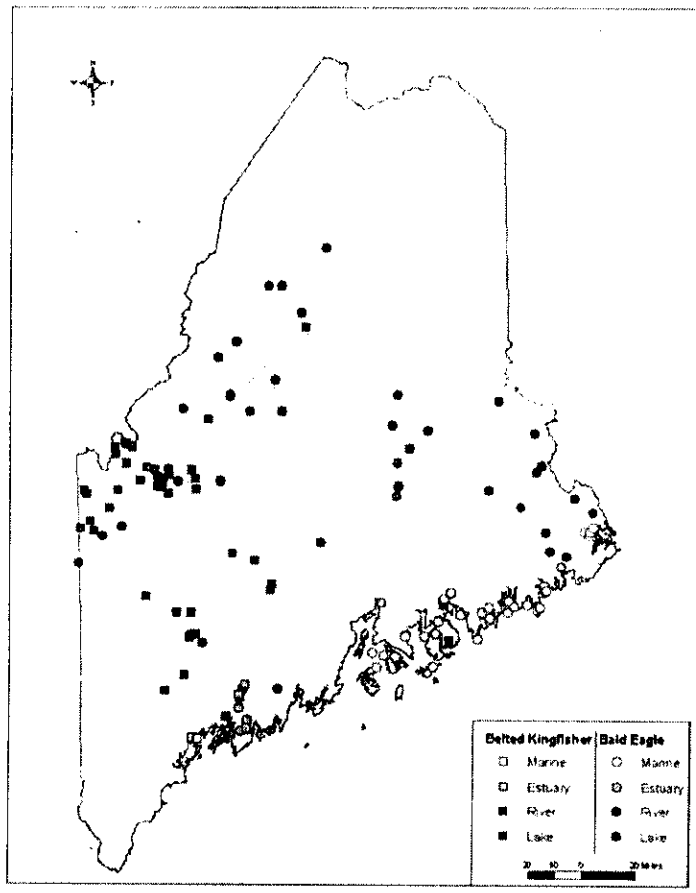

Figure 2. Distribution of sampling effort by habitat type for the belted kingfisher and bald eagle in Maine.

All tissue data represent analysis of total Hg on a wet weight (ww), in the case of feathers, fresh weight (fw), basis in μg/g (or ppm). Estimated values or ranges of the proportion of MeHg in a particular tissue are cited for each within the Discussion section. The term juvenile means young-of-year birds and adults signify individuals at least one year of age. Latin names for those species within our Hg data sets are provided in Appendix 1.

Common loon blood and egg Hg sampling locations were converted into an ESRI ArcView point shapefile (i.e., formatting georeferenced parameters in a way that can be used by spatial software). Egg Hg values were converted to adult female blood equivalency with $y = 1.5544x + 0.2238$ (Evers et al., 2003). A six latitudinal minute by six longitudinal minute polygon grid created in Coordinate Grid Maker 2.29 was layered on the loon data. The 6-min interval was chosen as the best resolution to balance local and regional trends. The loon Hg shapefile was spatially joined to the grid polygon where the arithmetic mean of all the points falling within a grid cell was calculated. These global means were then displayed in 1.0 μg/g (ww) intervals.

Laboratory methods

The data utilized in this compilation were generated at a number of laboratories over a period of several years. Although there were some

differences in sample preparation and analytical methods, all analyses included quality control (QC) samples to allow evaluation of accuracy and precision, and all laboratories utilized atomic absorption spectroscopy to measure Hg concentrations.

Sample types collected and submitted to the laboratories for analysis primarily included avian blood, feathers, and eggs. Blood samples were either in sealed capillary tubes or in glass or plastic vacutainer-type collection tubes. Samples that were severely clotted were not analyzed unless the entire sample could be removed from the collection tube.

Feather samples were either analyzed whole or as subsamples following homogenization. Aliquots of feathers were obtained by reducing individual feathers to small pieces with either stainless steel scissors or a Spex 6800 cryomill.

Egg samples generally required homogenization; a task that was sometimes complicated by the egg samples that were fully formed. Egg samples that were largely soft tissue were homogenized by either a Tissuemiser or a small food processor/blender prior to subsampling. Eggs containing hard parts and feathers were homogenized with a blender or with a Spex 6800 cryomill. Only loon eggs were corrected for moisture loss

Most blood, feather, and egg samples required digestion prior to analysis. This was accomplished by following a procedure similar to EPA 245.6, in which nitric and sulfuric acids were used in conjunction with potassium permanganate and potassium persulfate to solubilize the tissue and convert any bound Hg to the free Hg^{2+} ion (Lobring and Potter, 1991). Prior to analysis, excess $KMnO_4$ was reduced with hydroxylamine hydrochloride and the samples were made to volume with deionized water.

Analysis of digest solutions was based on the "cold vapor" atomic absorption spectroscopy method first introduced by Hatch and Ott (1968). Using either a manual or automated approach, Hg^{2+} in solution was reduced to Hg^0 with $SnCl_2$, the Hg^0 was transferred to the gas phase, and the Hg^0 -containing gas was swept into an atomic absorption cell. Mercury levels were determined by comparing sample absorbance peak heights with those of calibration standards.

A subset of samples was analyzed by a direct determination method that did not require sample digestion (EPA 7473) (U.S. EPA, 1998). A homogenized, dry sample was placed in a tared nickel boat, weighed, and then placed into a tube furnace. A stream of O_2 assisted in sample combustion and carried free or organic-bound Hg species through a heated catalyst and onto a gold trap where the free Hg^0 was collected. When the sample had been combusted for a sufficient length of time, the gold trap was heated and the released Hg^0 was carried through a pair of atomic absorption cells where it was measured. This method required samples that were particularly well-homogenized because only a small sample mass could be accommodated in the nickel boats.

Each batch of samples processed and analyzed was accompanied by a number of QC samples, including a method blank, spiked blank, certified reference material, duplicate sample, and spiked sample. Typical detection limits for data presented here were 0.0025 μg/g (ww). Precision as measured as relative percent difference of duplicate pairs was approximately 85% and accuracy as measured by recovery of certified reference materials and spiked samples was 80%.

Statistical analysis

Mercury concentrations are expressed as arithmetic means with standard deviations (SD) in the tables and geometric means with variation expressed as standard error (SE) in figures. Arithmetic means and SD are provided for comparative purposes with published literature. Because sample sizes were regularly small and were therefore not normally distributed, statistical analysis was conducted on the exponentiated value of the mean of the log-transformed values. Log-transformed data were normally distributed based on normal probability plot residuals. Homoscedasticity was checked with Bartlett's test, which is sensitive to the normality assumption. JMP software (SAS Institute Inc., 2001) was used to perform statistical analysis. Hypotheses were tested using one-way analysis of variance (ANOVA). Testing was followed by post-hoc tests using Tukey–Kramar honestly significant different (Tukey's test) if the ANOVA demonstrated significant differences (Zar, 1999). JMP's Tukey's test output

did not include actual probability values and instead indicated significance when numbers were positive. Therefore, only probability values "less than" and "greater than" 0.05 are shown in the Results section. Student's *t*-tests were used when comparing paired data sets. A non-parametric test, the Kruskal–Wallis One-Way ANOVA, was used in some cases to compare multiple independent groups. JMP software corrected for inequity of unbalanced data sets. We used an alpha of 0.05 for our level of significance.

Results

A total of 4,769 Hg concentrations representing 38 species and six tissue types are recorded within the NSRC avian database (Appendix 1–3; Fig. 1). Samples were collected between 1969 and 2003 with the majority (> 84%) from 1995 to 2003. Six factors were identified as having significant influence on the interpretation of avian Hg levels. The NSRC data set was used to demonstrate how these factors influence Hg exposure.

Influences of tissue type

Mercury data collections totaled 2,158 blood, 943 egg, 281 muscle, 1,100 feather, 239 liver, and 48 kidney samples (Appendix 1). Approximate respective inter-tissue comparative ratios based on blood for common loons breeding in northeastern North America were 0.4:1:2:6:15 (egg:blood:muscle:feather:liver). For a site-specific subset of Hg exposure data (south-central Quebec, New England and Canadian Maritimes), there were no significant geographical differences among tissue ratios ($p > 0.05$; with the exception of blood) (Fig. 3). Muscle Hg levels among eight waterfowl species were categorized by four major foraging guilds during the breeding season and indicated significant differences between piscivores versus each of the other three foraging guilds and insectivores versus herbivores (Fig. 4).

Intra-and inter-tissue relationships were strongest in the following three pairings: (1) adult and juvenile blood, (2) adult female blood and egg, and (3) juvenile feather and blood. Data analyzed were based on sampling efforts that represented pairings from the same breeding territory (i.e., each pair of adult and juvenile blood Hg levels in tree swallows was from the same nesting box). Paired adult-juvenile blood Hg levels in common loons had a significant relationship ($r^2 = 0.63$, $p < 0.01$) as they did for tree swallows ($r^2 = 0.74$, $p < 0.01$). Paired adult female blood and egg Hg levels were significantly related in loons ($r^2 = 0.79$, $p < 0.01$) (based on Evers et al., 2003) and tree swallows ($r^2 = 0.49$, $p < 0.01$). Paired eaglet feather and blood Hg levels were significantly related ($r^2 = 0.67$, $p < 0.01$) (based on Welch, 1994).

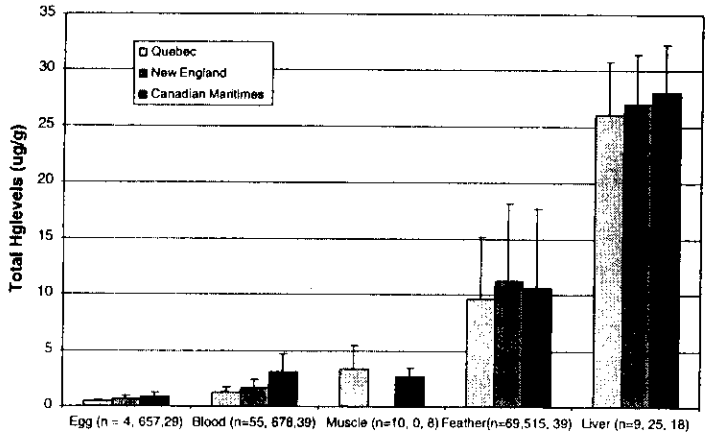

Figure 3. Comparison of geometric mean ± SE of Hg levels in four tissue types for adult common loons breeding in south-central Quebec, New England, and the Canadian Maritimes (n = number of respective samples by region). The arithmetic mean ± SD of Hg in liver is used for comparative purposes with the literature. Liver Hg values in New England are from Pokras et al. (1992).

Age as a factor

Five species of birds were used to demonstrate relationships in blood Hg levels between juveniles (< 2 months of age) and adults (> 1 year of age) (Fig. 5). Two species were insectivores (tree swallow and song sparrow) and three species were piscivores (common loon, common merganser, and belted kingfisher). While adult tree swallows had significantly higher blood Hg levels than nestlings ($p < 0.05$), adult song sparrows did not have significantly different blood Hg levels than their fledged young ($p > 0.05$). Significantly higher blood Hg levels in adults versus juveniles were found in all three species of piscivorous birds ($p < 0.05$). Ratios of adult-juvenile Hg levels were: song

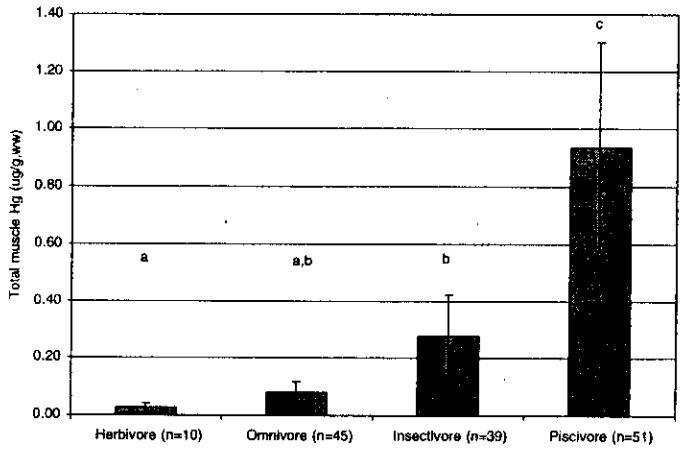

Figure 4. Comparison of geometric mean ± SE of Hg levels in muscle among four foraging guilds of waterfowl (n = number of samples). Means not sharing a common letter are significantly different ($p < 0.05$). Waterfowl species represented by foraging guild are: herbivores – Canada goose; omnivores – mallard, American black duck, green-winged teal, and ring-necked duck; insectivore – common goldeneye; and piscivore – hooded merganser and common merganser.

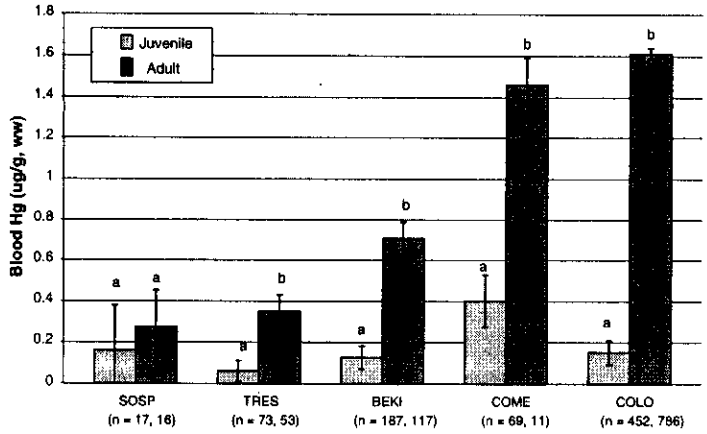

Figure 5. Comparison of geometric mean ± SE of blood Hg levels of five species for two age classes (n = number of juvenile samples, number of adult samples). Means not sharing a common letter within a given species are significantly different ($p < 0.05$). Species codes are: SOSP, song sparrow; TRES, tree swallow; BEKI, belted kingfisher; COME, common merganser and; COLO, common loon.

sparrow 1.7:1, common merganser 3.6:1, tree swallow 5.9:1, belted kingfisher 5.6:1, and common loon 10.6:1.

Gender as a factor

Three species were used to demonstrate a relationship in blood Hg levels between male and female adults (Fig. 6). Blood Hg levels in male common loons were significantly higher than females ($p < 0.01$). In belted kingfishers and tree swallows, there was no significant difference between male and female blood Hg levels ($p > 0.05$).

Aquatic habitat comparisons

Data for the belted kingfisher and bald eagle showed relationships in MeHg availability for four major aquatic habitat categories: marine, estuarine, riverine, and lake. Only individuals sampled in Maine were used for an analysis of inter-habitat differences (Fig. 2). Both species exhibited an increasing trend in which mean blood Hg levels in marine < estuarine < riverine < lake (kingfishers, Fig. 7 and eagles, Fig. 8). Adult blood Hg levels were significantly higher in kingfishers foraging on lakes and rivers versus marine habitats ($p < 0.05$), but not different in rivers versus estuaries ($p > 0.05$). Eaglet blood Hg levels were significantly higher from nests along lakes versus those adjacent to marine ($p < 0.05$) and estuarine ($p < 0.05$) habitats. Blood Hg levels in eaglets from nests along rivers were significantly higher than those along marine habitats ($p < 0.05$).

Geographic differences

Based on a standard species (common loon) and tissue types (blood and egg) and a large sample size (n = 1,882), spatial heterogeneity in MeHg availability was demonstrated across northeastern North America (Fig. 9). The proportion of mean blood and converted egg Hg concentrations (see "Source data sets" in Methods section) within a six latitudinal minute by six longitudinal minute cell grid (n = 300 cell grids) was 19.7% for 0–1 μg/g, 45.6% for 1–2 μg/g, 20.7% for 2–3 μg/g, 8.7% for 3–4 μg/g, and 5.3% for >4 μg/g.

Variation in species

Three sites provided an opportunity to compare Hg levels in multiple species within the same area (which avoids confounding factors related to geographical differences): two examples were on lakes and one was on a river. On Aziscohos Lake, eggs of five species (three piscivores, one insectivore, and one herbivore) were compared (Fig. 10).

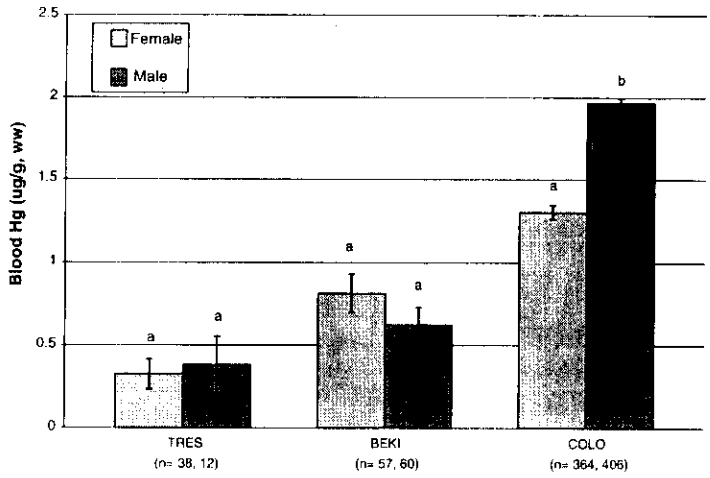

Figure 6. Comparison of geometric mean + SE of blood Hg levels of three species by sex (n = number of female samples, number of male samples). Means not sharing a common letter within a given species are significantly different ($p < 0.05$). Species codes are: TRES, tree swallow; BEKI, belted kingfisher and; COLO, common loon.

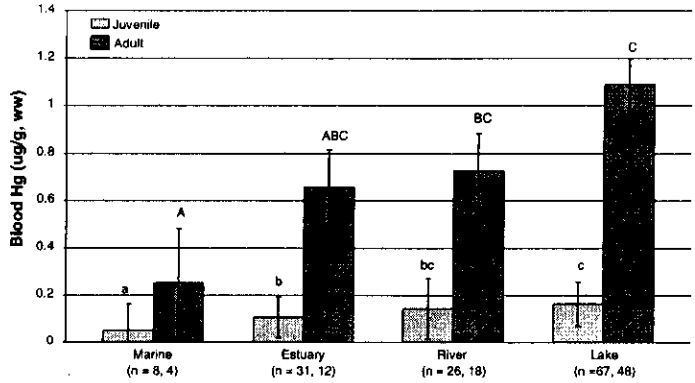

Figure 7. Comparison of geometric mean ± SE of blood Hg levels for juvenile and adult belted kingfishers in Maine among four major aquatic habitat types (n = number of juvenile samples, number of adult samples). Means not sharing a common letter are significantly different ($p < 0.05$); comparisons are case sensitive.

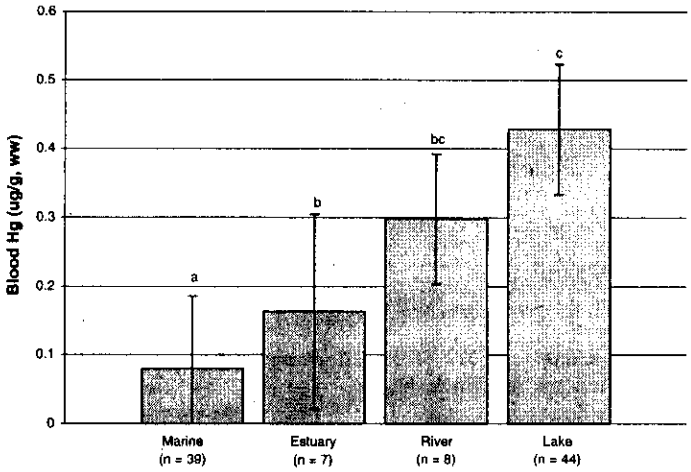

Figure 8. Comparison of geometric mean ± SE of blood Hg levels for juvenile bald eagles in Maine among four major aquatic habitat types (n = number of samples). Means not sharing a common letter are significantly different ($p < 0.05$).

Piscivore egg Hg levels were significantly higher ($p < 0.05$) than insectivores (tree swallows) and herbivores (wood duck). Larger piscivores tended to have higher egg Hg levels than smaller piscivores (i.e., common loon > common merganser > hooded merganser). Similar patterns were documented on Flagstaff Lake in egg Hg levels (common loon > common merganser > belted kingfisher) (Fig. 11).

Blood Hg levels were compared for 11 insectivorous birds from the Sudbury and Charles Rivers (Fig. 12). There were no significant differences in blood Hg levels between the rivers for four of the most common passerines sampled at each site (Kruskal–Wallis one-way analysis of Variance; $p = 0.65$). Age classes were combined because song sparrow blood Hg levels were not significantly different between breeding adults and nestlings (Fig. 5). Generally, blood Hg levels increased with body weight. For seven selected songbirds, there was a significant correlation between mean weight and blood Hg levels ($r^2 = 0.72$,

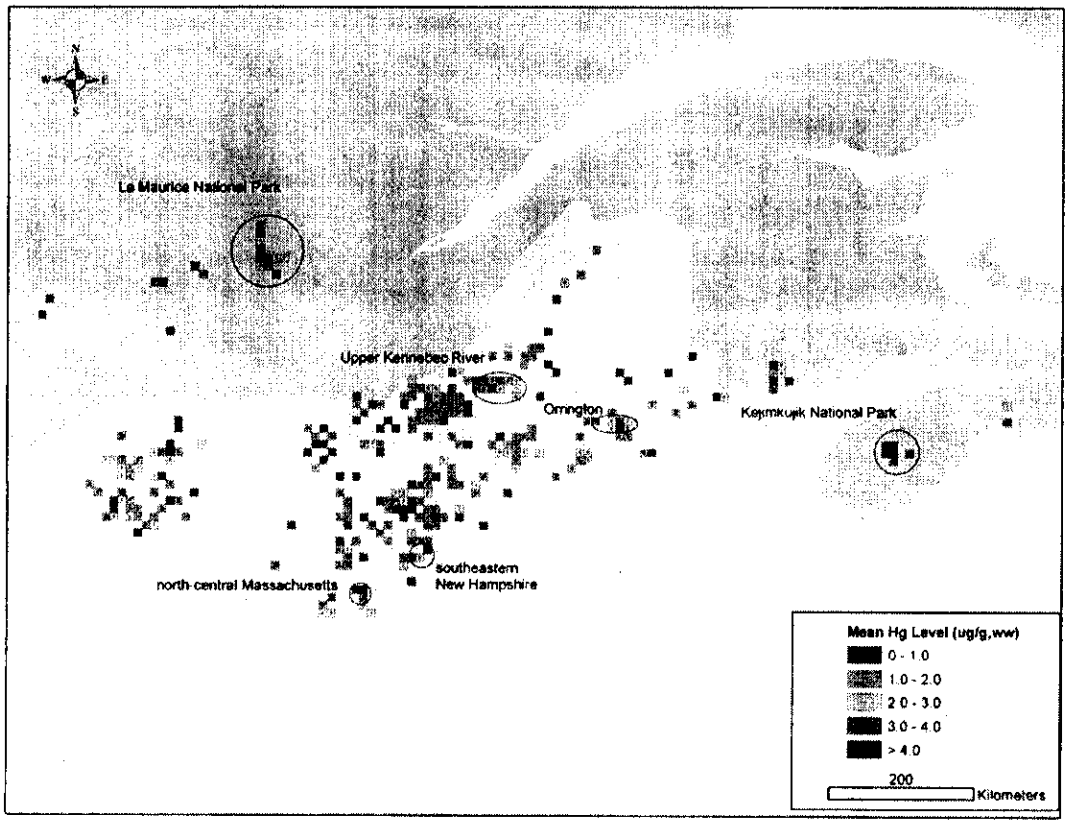

Figure 9. Geographic distribution of mercury levels in adult common loon blood and blood equivalents based on eggs, 1993–2003.

$p = 0.02$, $y = 0.0162x + 0.0054$). Granivorous songbirds such as the American goldfinch had blood Hg levels significantly lower than insectivorous songbirds ($p < 0.05$).

Discussion

Selecting the correct tissue

The pharmacokinetics of MeHg and total Hg (how both forms are distributed throughout the body) are fairly well known in birds; their understanding provide insights toward selecting the correct tissue for meeting specific research or monitoring objectives. Ingestion of dietary MeHg appears to be readily absorbed into the blood (83% in common loons, Fournier et al., 2002) and is thereafter distributed to various body tissues, namely the liver, kidney, brain, spleen, and muscle. Some tissues, such as the liver, are terminal endpoints where MeHg is largely unavailable for remobilization, whereas MeHg deposited in muscle tissue is available and remobilizes during feather molt. Blood and feather Hg samples are generally taken nonlethally. Although blood can be taken immediately after death through cardiac puncture (Henny et al., 2002), it cannot be taken through venipuncture after death for reliable comparison with living bird blood Hg levels (because of rapid and nonlinear moisture loss).

Mercury concentrations in avian tissues can indicate different (1) modes of toxic action, (2) MeHg and total Hg composition, (3) exposure timeframes, and (4) elimination abilities. Six tissues routinely used for determining Hg exposure in birds are described in our data set.

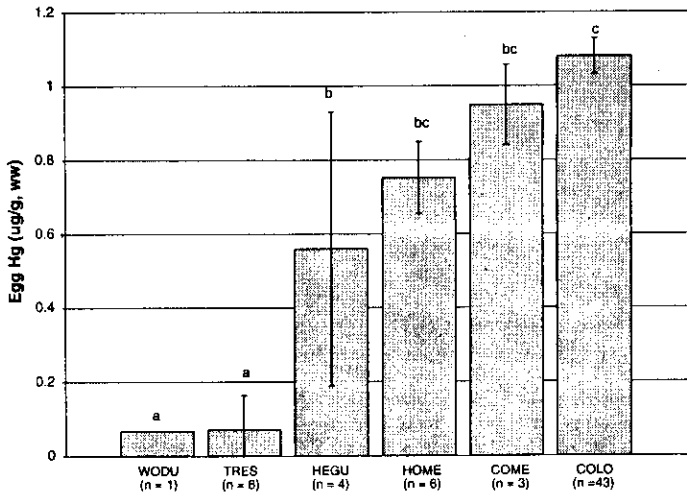

Figure 10. Comparison of geometric mean ± SE of egg Hg levels among species on Aziscohos Lake, Maine (n = number of samples). Means not sharing a common letter are significantly different ($p < 0.05$). Species codes are: WODU, wood duck; TRES, tree swallow; HEGU, herring gull; HOME, hooded merganser; COME, common merganser and; COLO, common loon.

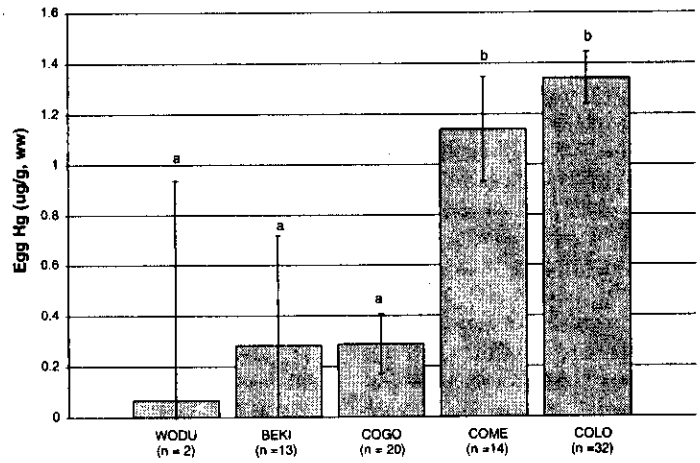

Figure 11. Comparison of geometric mean ± SE of egg Hg levels among species on Flagstaff Lake, Maine (n = number of samples). Means with different letters are significantly different ($p < 0.05$). Species codes are: WODU, wood duck; BEKI, belted kingfisher; COGO, common goldeneye; COME, common merganser and; COLO, common loon.

Tissue interpretation

Blood is the best tissue for evaluating short-term dietary uptake. Mercury in blood is primarily MeHg (> 95%) in both piscivores (Fournier et al., 2002) and insectivores (Rimmer et al., 2005). The half-life of MeHg in the blood of chicks undergoing feather molt was three days in common loons (Fournier et al., 2002) and 5–6 days in Cory's shearwaters (Monteiro and Furness, 2001). In non-molting adults, the half-life of MeHg in the blood was greater: for Cory's shearwater (*Calonectris diomedea*) the half-life was 40–60 days (Monteiro and Furness, 2001) and for the mallard was 74 days (Heinz and Hoffman, 2004). In our study, adult loon blood Hg levels, which were

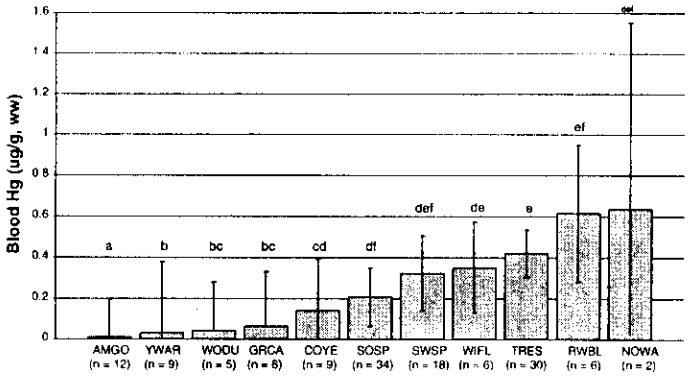

Figure 12. Comparison of geometric mean ± SE for blood Hg levels (adult and juvenile combined) among species on or near the Sudbury and Charles Rivers, Massachusetts (n = number of samples). Means not sharing a common letter are significantly different ($p < 0.05$). Species codes are: AMGO, American goldfinch; YWAR, yellow warbler; WODU, wood duck; GRCA, gray catbird; COYE, common yellowthroat; SOSP, song sparrow; SWSP, swamp sparrow; WIFL, willow flycatcher; TRES, tree swallow; RWBL, red-winged blackbird and; NOWA, northern waterthrush.

collected 60–120 days post-arrival to the breeding lake, were strongly correlated with prey fish Hg levels and therefore primarily reflected uptake of dietary Hg from the breeding lake (Fevold et al., 2003; Evers et al., 2004, Burgess and Hobson, in press; Champoux et al., in press).

As in blood, egg Hg levels are primarily in the MeHg form: in loons it was >95% (Scheuhammer et al., 2001; Evers et al., 2004), in seabirds it is >90% (Fimreite et al., 1974). Because female blood Hg levels are highly correlated with egg Hg levels for the common loon (Evers et al., 2003), eggs and their outer membranes are also pertinent tissues for predicting Hg exposure within a bird's breeding territory (Heinz and Hoffman, 2003). How predictive egg Hg levels are for the breeding territory depends on amount of time the female spent within the territory prior to egg-laying and Hg body burden levels accumulated during the winter and migration. Generally, piscivores arriving from marine overwintering areas have been exposed to lower MeHg availability than levels found on freshwater aquatic systems (Fig. 7, 8). Rapid equilibrium of dietary MeHg uptake and blood MeHg levels thereafter plays an important role in forming a strong MeHg relationship between eggs and breeding-season blood levels. The strength of this relationship is also impacted by intraclutch differences in Hg levels. In common loons, intraclutch variation between two eggs was 25% (Evers et al., 2004), in larids it averaged 39% between the first and second eggs (Becker et al., 1994), and in common merganser clutches of >10 eggs we sometimes found variations of one order of magnitude (although nest parasitism in cavity-nesting ducks is common and it unknown if merganser clutches represent more than one female). A standardized comparison of egg Hg levels among locations therefore requires knowledge of egg laying order.

Virtually all Hg in a feather is MeHg (Thompson and Furness, 1989) and is sequestered for long time periods allowing retrospective analysis (Frederick et al., 2004). Feather Hg reflects blood Hg levels at the time of molt (Bearhop et al., 2000), however, if MeHg is depurated in the muscle tissue (as is the case for individual birds with a high dietary MeHg uptake), it is available for remobilization. Therefore, feather Hg levels reflect both site-specific dietary uptake of MeHg and body burden. Feather Hg generally reflects 70–93% of the muscle MeHg burden (Burger, 1993); therefore, there can also be chronic bioaccumulation of MeHg, particularly for highly exposed individuals (i.e., where MeHg ingestion exceeds elimination). This attribute makes feather Hg levels a relevant tissue for evaluating chronic body burdens, particularly when considering the stability of MeHg in the feathers (Appelquist et al., 1984). However, individual variation in physiological response to

Hg (Bearhop et al., 2000), as well as the broad differences in inter-species pharmacokinetics, requires careful evaluation of risk.

Four internal tissues are commonly used for Hg exposure investigations: brain, liver, kidney and muscle. Although MeHg crosses the blood-brain barrier and can have significant impacts on brain functions, brain tissue is best harvested from relatively fresh carcasses and therefore is a more difficult tissue to use in field studies. It is more commonly used for analysis in experimental studies (Heinz, 1975; Finley et al., 1979; Scheuhammer, 1988).

Liver is one of the more commonly analyzed internal tissues for Hg in birds (Sundlof et al., 1994; Augspurger et al., 1998; Pokras et al., 1998; Cohen et al., 2000). Liver and kidney filter toxins such as MeHg and effectively demethylate MeHg using selenium (Se) bonds that form a nontoxic Hg-Se protein complex (Stoewsand et al., 1974). Scheuhammer et al., (1998a) found a nearly 1:1 molar ratio of Hg:Se in the liver and kidney of common loons and common mergansers. He also demonstrated that the proportion of MeHg in the liver and kidney declined as total Hg concentrations increased (i.e., liver and kidney total Hg levels were independent of MeHg concentrations). Therefore, determining levels of currently toxic Hg in the liver and kidney requires analysis of MeHg concentrations, while concentrations of Se-bound inorganic Hg provide an indication of past MeHg exposure.

The "7:3:1 rule" is an often-used conversion factor for liver (ww), feather (fw), and muscle (ww) tissue Hg concentrations (Appelquist et al., 1985). Although Thompson et al., (1990) demonstrated the weakness of this conversion approach, these tissues in common loons of three different regions in northeastern North America follow the "7:3:1 rule" (Fig. 3).

Unlike the liver and kidney, muscle Hg levels generally have proportionally higher levels of MeHg (80–100%) in the common loon and common merganser (Scheuhammer et al., 1998a). Muscle tissue Hg levels are generally examined in waterfowl (Pearce et al., 1976; Braune et al., 1999; Cohen et al., 2000), in part, to determine potential human health risks. Our data set supports other findings that muscle Hg levels are generally less than liver and kidney (Gardiner, 1972; Gochfeld, 1980) and that piscivorous waterfowl have muscle Hg levels significantly greater than other foraging guilds of waterfowl species (Fig. 4).

Age affects Hg exposure

A potential limitation of using birds as an indicator of Hg is the inability to identify individual age once breeding begins. The knowledge of a bird's age is critical for evaluating Hg bioaccumulation. Marking techniques, such as uniquely numbered or colored leg bands, provide a reliable method for tracking individual age and removing this limitation. Such techniques have been used successfully to evaluate time relationships with Hg. In cases where MeHg ingestion exceeds elimination, feather Hg levels increase with age (Evers et al., 1998; Rimmer et al., 2005). Scenarios where individuals can depurate and demethylate ingested MeHg at a similar annual rate of ingestion lack a positive correlation with increasing age and feather Hg levels (Furness et al., 1990; Thompson et al., 1991; Gochfeld et al., 1996; Donaldson et al., 1997; Fevold et al., 2003).

While bioaccumulation of MeHg can be a critical interpretive factor related to age in situations with high Hg exposure, there is also a common pattern for adult blood Hg levels to exceed those of unfledged juveniles in areas with even low Hg exposure (Fig. 5). Burger and Gochfeld (1997) documented adult Franklin's gull (*Larus pipixcan*) and herring gull blood Hg levels to be significantly greater than juveniles from the same colony of the same year. Differences in Hg levels between age classes (juvenile vs. adult) is dictated by (1) stage of juvenile feather molt and (2) partitioning of forage base by size of potential prey.

A major depuration route for MeHg is via the feather (Crewther et al., 1965), and therefore feathers are a useful indicator for monitoring Hg body burdens (Burger, 1993). However, the interpretation of feather Hg levels requires an understanding of feather-molt chronology (Furness et al., 1986). Blood and feather MeHg levels are highly correlated when blood is sampled during feather molt. Birds have the ability to rapidly transfer dietary uptake of MeHg from red blood cells to growing feathers (Fournier et al., 2002; Kenow et al., 2003). The physiological capacity of birds to process MeHg this way appears to be great. Fournier et al., (2002)

found loon chicks, experimentally dosed with MeHg concentrations substantially greater than those found in nature, were still able to effectively depurate much of the MeHg into emerging feathers. When juvenile feather molt ends, blood MeHg levels thereafter increase (Spalding et al., 2000a; Fournier et al., 2002). This ability to rapidly transfer blood MeHg into growing feathers partly accounts for the significant difference in blood Hg levels between adults and juveniles prior to fledging. Our data indicate that this is relevant for piscivorous and insectivorous birds. For the song sparrow, blood Hg levels of recently fledged young when compared with locally breeding adults, demonstrated no significant difference between the age classes. However, difference of age ratios among species (Fig. 5) indicates Hg differences between age classes are also dictated by other factors.

Generally, prey choice differs between adults foraging for themselves versus for their young. For example, the size of fish prey selected by juvenile common loons increases as they grow larger (Barr, 1996). We found adult blood Hg levels of piscivore species were 3.6 to 10.6 times higher than those in unfledged young (Fig. 5) and that variation suggests that adults are foraging on prey that are larger and have higher levels of Hg. In agreement, Burgess and Hobson (in press) found that adult loons fed at a higher trophic level (as indicated by stable-nitrogen isotope ratios) and had higher blood Hg levels than did juvenile loons. These differences in blood Hg and trophic levels were related to differences in body weight in the loons. Blood sampling for belted kingfishers and tree swallows typically occurs when the adults are 6 times the weight of the juveniles, while in common loons, adults are sampled when they are usually 10 times the weight of juveniles. Conversely, common mergansers are usually sampled when the adult females are relatively similar in size with the young. Such rough correlative relationships across age classes between body weight and prey-size selection are likely contributing to age class differences in Hg exposure.

Gender affects Hg exposure

Although most studies indicate differences in Hg levels between male and female birds are not significant (Burger, 1993; Burger, 1995; Gochfeld et al., 1996), there are exceptions (Hoffman and Curnow, 1979; Braune and Gaskin, 1987; Donaldson and Braune, 1999). When there are differences in Hg levels between sexes they can be dictated by (1) depuration of Hg in eggs, (2) sexual dimorphism, and (3) niche partitioning of the forage base. Although depuration of MeHg in eggs is an important mechanism for elimination, derivation of MeHg from serum proteins and a steady-state equilibrium with dietary uptake of MeHg likely compensates for the loss of MeHg from the body burden within weeks (Furness and Greenwood, 1993) or possibly days (Kambamandi-Dimou et al., 1991). Differences in blood Hg levels between sexes of adult loons sampled >60 days after eggs are laid regularly demonstrates other factors are involved. Choice of prey items is likely the primary factor dictating differences in Hg levels between sexes. Levels of MeHg in prey items vary according to species, trophic status, age, size, and habitat associations (Wiener and Spry, 1996).

Based on comparisons of three species, the loon exhibited significant differences of blood Hg between sexes. Loon Hg levels were also greater in liver for males vs. females (Pokras et al., 1998). Common loons are sexually dimorphic. On average, males are 21% larger than females (Evers, 2005). The larger males apparently forage on larger prey fish based on the correlative strengths between favored prey fish Hg levels (yellow perch, *Perca flavescens*) and their blood Hg levels (Evers et al., 2004). Larger fish generally have higher Hg levels than smaller fish of the same species from the same location (Weiner and Spry, 1996; Drysdale et al., in press; Kamman et al., 2005). Therefore, blood Hg levels are higher in male common loons because they are foraging on larger fish.

In general, blood Hg differences between male and female belted kingfishers are not significant, however, within individual pairs Hg levels are typically significantly different. Because males and females are relatively similar in size (females tend to be slightly heavier), prey size is likely not the driving factor; rather males and females partition foraging niches (Albano, 2000).

Hg patterns in aquatic habitats

Aquatic systems are one of the more at-risk ecosystems for MeHg bioavailability because one

of the better-known methylating organisms, sulfate-reducing bacteria, inhabit this environment (Gilmour et al., 1992). To adequately compare MeHg bioavailability across four major aquatic habitat types requires a standard species, age class and sampling tissue. Both the bald eagle and belted kingfisher fit these criteria. A subset of the blood Hg data from both species in Maine indicates interspecies agreement that MeHg availability increases from marine to estuarine to riverine to lake ecosystems. Because atmospheric deposition of Hg is relatively uniform across the Maine study area (VanArsdale et al., 2005), with some significant local exceptions, interpretation of the hydrological and biogeochemical factors influencing Hg methylation and availability and their relationships with bird blood Hg levels is presented.

Although marine systems are well known for their elevated biotic MeHg levels, those levels primarily represent long-lived species with top trophic status (e.g., swordfish and shark species). A standard comparison between freshwater and in-shore marine systems documents the latter has significantly lower MeHg availability. In-shore marine systems appear to be more effective in diluting MeHg production versus freshwater systems, although in-shore habitats geochemically greatly vary in MeHg production (Hammerschmidt and Fitzgerald, 2004). Estuaries are dynamic communities that are influenced by tidal actions and varying volumes of fresh and salt water. They are generally hydrologically heterogeneous landscapes that have less of an ability to dilute Hg inputs than marine systems. Although tidal exchanges do regularly provide an important flushing mechanism that lowers MeHg availability (Lamborg et al., *In Press*), tidal responses vary in magnitude daily, weekly, and monthly. Methlymercury availability therefore varies tremendously within and between estuaries (Shriver et al., 2002).

Based on our analysis, the ability of inorganic Hg to be converted to MeHg and become available to biota is greater in freshwater versus saltwater habitats. Gariboldi et al., (1998) also documented prey items were higher in Hg from freshwater versus saltwater habitats based on sampling efforts with the wood stork (*Mycteria americana*). Riverine habitats tend to have higher levels of MeHg availability than estuaries, but tend to have lower levels when compared to lakes. Comparisons of MeHg availability between adjoining riverine and lake habitats based on crayfish, fish, and birds consistently show greater Hg exposure on lakes (Fimreite, 1974). Flushing abilities within riverine systems are a driving force for these differences.

Therefore, in coastal regions, MeHg exposure arising from atmospheric Hg deposition is generally greatest in piscivorous birds foraging on freshwater lakes. Lake hydrology and biogeochemistry largely determine the degree of aquatic MeHg exposure. Lakes with low pH (< 6.3, Meyer et al., 1995; Burgess and Hobson, in press), large areas of scrub-shrub and emergent wetlands (Kramar et al., 2005), and large areas of exposed shoreline substrate of organic or sandy soils that are frequently inundated and dried through the summer and fall (i.e., reservoirs; Evers and Reaman, 1998) are predictive of elevated blood Hg levels in the common loon. Although newly created reservoirs are well known for their ability to enhance MeHg production and availability through the decomposition of vegetation (Jackson, 1988; Lucotte et al., 1999; Gerrard and St. Louis, 2001), this phenomenon is generally viewed as short-lived (i.e., < 10 years in secondary consumers) (Lucotte et al., 1999). Some reservoirs have longer lasting abilities to enhance Hg methylation and have the potential to be some of the highest risk aquatic habitats (Evers et al., 2004).

Geographic differences exist

There are continental patterns in the availability of MeHg. Long-term sampling efforts across North America indicate a significant west to east trend exists with northeastern North America exhibiting the highest levels (Evers et al., 1998, 2003; Scheuhammer et al., 2001). Significant within-region differences are primarily driven by hydrological and biogeochemical factors and point source influences. The collection of > 1,800 blood and egg Hg levels for the common loon across New England, New York and eastern Canada enabled us to effectively evaluate areas of greatest concern (Fig. 9). Clusters of elevated MeHg availability were found in the western Adirondack Mountains of New York, north-central Massachusetts,

southeastern New Hampshire, western mountains of Maine, and a small area east of Orrington, Maine in the United States (Fig. 9). In Canada, areas with high MeHg exposure were in eastern Ontario, south-central Quebec and southern Nova Scotia. There did not appear to be smooth spatial trends in loon Hg levels across northeastern North America, as highly elevated Hg levels were scattered among low Hg concentrations in almost every region sampled.

In the Adirondack Mountains and eastern Canada, clusters of elevated MeHg availability were likely related primarily to lake acidification. It is well established that lakes with low pH contain fish with higher levels of Hg than same-size and species of fish in lakes with more circumneutral pH levels (Wiener et al., 1990; Winfrey and Rudd, 1990; Drysdale et al., in press). Oligotrophic lakes in eastern Canada and parts of New England and New York are susceptible to increased rates of anthropogenically derived sulphur deposition (i.e., acid rain) (Driscoll et al., 2001). Although there is evidence of declining levels of atmospheric input of sulfur dioxide, base cation levels are lowered in many systems where responses in lake pH levels are lagging behind predictive models. Therefore, these acidified lakes continue to be a cause of concern for their ability to enhance MeHg productivity. Associations between lake acidity, fish Hg levels and lower common loon productivity have been observed in the U.S. and Canada (Meyer et al., 1998; Burgess et al., 1998a). Scheuhammer and Blancher (1994) predicted up to 30% of lakes in central Ontario have the potential to adversely impact common loon productivity.

Other areas of concern are related to topography and lake hydrology (e.g., western mountains of Maine) and point sources (both airborne and waterborne). Airborne sources in southern New England appear to contribute to greater-than-expected loon Hg levels in southeastern New Hampshire (Evers, 2001) and Orrington, Maine. Waterborne point sources are well known in eastern Massachusetts, such as on the Sudbury River. There, investigations associated with the Nyanza Superfund Site have documented associated Hg contamination >25 km downstream from the point source (Wiener and Shields, 2000).

Species Hg exposure patterns

Differences in Hg levels among species are dictated by trophic level and availability of MeHg (i.e., aquatic vs. terrestrial and marine vs. freshwater; low exposure vs. high exposure). Trophic structure is a primary driver of variability in MeHg biomagnification (Cabana et al., 1994). Recent evidence indicates that the trophic status of an aquatic vertebrate is based primarily on the complexity, both longitudinal and vertical, of the planktivorous community (Chen et al., 2005). Methylmercury can biomagnify several orders of magnitude in aquatic ecosystems. For example, the average bioconcentration factor for the common loon in Maine lakes is 1.37×10^6 (based on unfiltered water for total Hg) (Evers et al., 2004).

The degree of MeHg biomagnification through aquatic-based food webs is the primary reason for the multitude of Hg studies on obligate piscivores. Particular emphasis has been placed on larger species for which trophic status is potentially greatest; such species include the common loon (Meyer et al., 1995, 1998; Burgess et al., 1998a, b; Evers et al., 1998, 2003; Scheuhammer et al., 1998b, 2001; Fevold et al., 2003; Burgess and Hobson, in press; Champoux et al., in press), bald eagle (Grier, 1974; Wiemeyer et al., 1984; Frenzel and Anthony, 1989; Bowerman et al., 1994; Anthony et al., 1999; Bowerman et al., 2002), osprey (Cahill et al., 1998; DesGranges et al., 1998), wading birds (Gariboldi et al., 1998; Bouton et al., 1999; Spalding et al., 2000b; Henny et al., 2002), and seabirds (Braune, 1987; Burger and Gochfeld, 1995; Monteiro and Furness, 1995, 1997; Braune et al., 2001). Other foodweb pathways important for MeHg transfer are generally of lesser concern because trophic status of the endpoint species is generally lower than piscivores. Benthic-based MeHg transfer through bivalves has been investigated using various diving species of waterfowl (Ohlendorf et al., 1986; Henny et al., 1991; Braune et al., 1999; Cohen et al., 2000; Wayland et al., 2002), while such transfer through macroinvertebrates (larval and adults) (Bishop et al., 1995; Wolfe and Norman, 1998; Reynolds et al., 2001; Gerrard and St. Louis 2002, Adair et al., 2003) and vegetation has also been described (Fimreite, 1974; Langis et al., 1999).

Elevated MeHg bioavailability in specific terrestrial ecosystems within northeastern North America has recently been documented. Montane environments without standing water appear to have the ability to generate MeHg. Rimmer et al., (2005) documented Bicknell's Thrush blood Hg concentrations for 21 mountain locations (arithmetic mean of 0.14 ± 0.08 μg/g, ww with a range of < 0.01 to 0.70 μg/g, ww) at levels similar to those found in many of the insectivorous songbirds sampled along rivers in Massachusetts (Fig. 12).

The comparison of multiple species within the same area and habitats, while using appropriate tissues and minimizing confounding factors (such as age class and sex), is the optimal approach for determining interspecies relationships of Hg exposure. Based on such past studies (Dustman et al., 1972; Fimreite, 1974; Langis et al., 1999) and our data sets, Hg exposure can be approximately predicted by foraging guilds. An all-purpose ranking from low to high Hg exposure for birds is: terrestrial herbivores, aquatic herbivores, terrestrial insectivores, benthivore-bivalves, benthivore-macroinvertebrates, small piscivores, and large piscivores. Exposure of Hg in scavengers and omnivores is broad and dependent on opportunistic food sources (Fimreite, 1974). Our ranking assumes MeHg availability is driven by atmospheric deposition and is not universal in application, because some habitats such as montane ones contain insectivorous birds that have Hg exposure greater or equivalent to piscivores.

Recommended bioindicators

We recommend species and tissue types that best indicate 12 targeted scenarios (Table 1) based on the analysis of our data set, the recommendations made by the working group (USEPA Hg Mason et al., 2005), species' ubiquitous within northeastern North America, and logistical feasibility. Identified indicator species are not universal and may be only relevant to the scenario posed. Many of our chosen bioindicators are also useful for determining MeHg effects through such endpoints as long-term reproductive success. For example, bald eagle breeding populations are used in Michigan (Bowerman et al., 2002) and common

Table 1. Summary of recommended avian bioindicators, age/sex class, and tissue type for 12 scenarios in freshwater, estuarine, and terrestrial systems in northeastern North America

Scenario	Species	Age/sex[1]	Tissue type
Comparison of major aquatic habitat types	Belted kingfisher	Adult & fledged young	Blood & egg[2]
	Bald eagle	Juvenile	Blood & feather
Lake > 25 ha	Common loon	Adult	Blood & egg
	Common merganser	Adult female	Blood & egg[2]
Lake < 25 ha	Common loon	Juvenile	Blood
	Hooded merganser	Adult female	Blood
River	Common merganser	Adult female	Blood & egg[2]
	Belted kingfisher	Adult & juvenile	Blood& egg[2]
	Tree swallow	Adult & juvenile	Blood & egg
Estuaries	*Ammodramus* sparrow spp.	Adult & fledged young	Blood
Emergent wetlands	American bittern	Adult	Blood & egg
	Virginia rail	Adult	Blood & egg
	Song sparrow	Adult & fledged young	Blood
	Red-winged blackbird	Adult & fledged young	Blood & egg
Shrub–scrub wetlands	Waterthrush spp.	Adult	Blood
	Swamp and Song sparrows	Adult	Blood
Montane areas	Bicknell's thrush	Adult & fledged young	Blood
Deciduous forest	Wood thrush	Adult & fledged young	Blood
Coniferous forest	*Catharus* thrush spp.	Adult and fledged young	Blood
Long-term risk in lakes	Common loon	Adult	Feather
Greatest risk in aquatic systems	Bald eagle	Adult	feather

[1] Juvenile = unfledged young which have yet to reach completion of feather molt and fledged young = young-of-the-year that have completed feather molt.

[2] When using egg tissue from these species, only use composite values for entire clutch to avoid wide intra-clutch variation.

loon breeding populations with color-marked individuals are monitored throughout New England (Evers et al., 2004) and Wisconsin (Meyer et al., 1998; Fevold et al., 2003).

Our selections are species- and genera-specific for illustrative purposes, but species with similar foraging requirements, behavior, and natural history patterns may be suitable surrogates; preferably, trophic status is similar. Evolving techniques in stable isotope analysis offer numerous applications to matching trophic status through analysis of tissues (e.g., blood, feather, egg, muscle, bone). Such techniques provide quantitative measures of trophic position (Hobson, 1993; Bearhop et al., 2000; Nisbet et al., 2002; Dominguez et al., 2003), dietary emphasis (i.e., freshwater vs. marine [Hobson, 1990; Mizutani et al., 1990; Bearhop et al., 1999], marine versus terrestrial [Hobson, 1987; Hobson and Sealy, 1991; Schmutz and Hobson, 1998]), contaminant bioaccumulation (Cabana and Rasmussen, 1994; Kidd, 1998; Atwell et al., 1998), and nutrient allocation to reproduction (Hobson et al., 1997; Hobson et al., 2000). Here in, scenarios and associated avian selections relate primarily to freshwater breeding habitats.

Selecting a standard species across multiple aquatic habitats, particularly between freshwater and saltwater ones, is difficult. The belted kingfisher is a ubiquitous species that is an obligate piscivore in all major aquatic habitats. As a burrow-nesting species, repeated access to young and adults is feasible for the kingfisher. Sampling efforts to determine site-specific exposure should focus on blood. Intraclutch variability in egg Hg levels appears to be high (Lane et al., 2004). Bald eagle pairs also commonly forage within all major aquatic habitats. Adults are difficult to capture, therefore, chicks are generally sampled to determine Hg exposure (Bowerman et al., 2002). Blood and breast feathers are the most common sampling tissues. Adult eagle feathers from the nest site can be useful for determining Hg exposure (Bowerman et al., 1994) and may reflect some of the highest Hg levels within an aquatic ecosystem. However, foraging habits of breeding pairs vary dramatically within and between breeding seasons, habitat type, and geographic area (Knight et al., 1990; Kozie and Anderson, 1991; Anthony et al., 1999). Although either fish or birds can comprise the majority of prey remains at eagle nests, Dominguez et al., (2003) found that stable-nitrogen isotope ratios showed little difference in trophic status among nests in Newfoundland.

The common loon is one of the better bioindicators of lake-specific MeHg availability as it has a top trophic position in the aquatic food web, is long-lived, and in most cases remains within its breeding territory for 4–6 months. Adult blood and egg Hg levels reflect dietary Hg exposure of breeding loons on lakes >25 ha. Territorial pairs occupying lakes <25 ha generally maintain and feed on more than one lake (i.e., multi-lake territories) (Piper et al., 1997). Because adult common loons with multi-lake territories rarely bring food items back to their natal lake to feed their young, blood Hg levels of juvenile loons best represent MeHg availability on their natal lake. Common mergansers are also obligate piscivores that can reflect lake-specific MeHg availability. Sampling efforts for adult females can be facilitated through the use of artificial boxes. Similar to kingfishers, intraclutch variation in egg Hg levels is high for mergansers and dictates individual egg or composite analysis (versus selecting one egg). Other high-trophic level piscivores that generally forage on lakes are not optimal lake-specific indicator candidates because (1) of their tendency to commonly use multiple waterbodies within their breeding territory, (2) they are difficult to efficiently capture and sample (e.g., great blue heron), and (3) they regularly prey on lower-trophic-level organisms such as benthic-feeding fish (e.g., osprey) and terrestrial birds and mammals (e.g., bald eagle). Instead, such species best represent MeHg availability at a watershed level. Double-crested cormorants *phalacrocorax-auritus* may be good indicators of multiple large lakes and other aquatic systems.

Determining mercury exposure in riverine habitats is most promising with the common merganser, hooded merganser, belted kingfisher, and tree swallow. The belted kingfisher is increasingly being used as an indicator for assessing Hg in riverine systems (Baron et al., 1997; Moore et al., 1999). Use of artificial nesting boxes on riverine habitat and experimental design interests for both piscivorous and insectivorous birds can be achieved with the hooded merganser and tree swallow.

Although most investigations of avian Hg exposure have focused on waterbodies, wetlands and strictly terrestrial habitats are increasingly being included during risk assessments. In emergent wetlands, insectivores best reflect MeHg availability. Larger-bodied insectivorous birds have greater Hg exposure than their smaller counterparts (Fig. 12). The Virginia rail (*Rallus limicola*) is a good indicator candidate because it is more insectivorous than the sora (*Porzana carolina*) and is more common and less limited by marsh size than the American bittern (*Botaurus lentiginosus*). Clapper rails (*Rallus longirostris*) in San Francisco Bay had greater body burdens of Hg than associated piscivorous birds, such as terns (U.S. Fish and Wildlife Service, 2003). The red-winged blackbird had some of the highest blood Hg levels of songbirds within a Massachusetts riverine wetland (Fig. 12). Although the red-winged blackbird and sparrow species (i.e., song and swamp) are granivores most of the year, during the breeding season they are obligate insectivores. Based on limited Hg data, both the Louisiana (*Seiurus motacilla*) and northern waterthrush may be insectivorous passerines at greatest risk in riverine habitats; waterthrushes forage specifically on aquatic organisms. Other relatively large-bodied, insectivorous passerines associated with northern aquatic systems, such as the rusty blackbird (*Euphagus carolinus*), may also be at risk; specific Hg-sensitive habitats are acidic headwater areas (Bank et al., 2005) draining recently logged coniferous catchments (Porvari et al., 2003).

Methylmercury availability in terrestrial insectivorous passerines is relatively unknown but a recent compelling study by Rimmer et al. (2005) indicates further investigations are needed. That study documented Hg levels in Bicknell's thrush and further comparison of the blood Hg level ranges show an overlap with those of eaglets; thereby indicating equivalent trophic status of a terrestrial-based insectivore with an aquatic-based piscivore. Recent investigations have demonstrated that MeHg is present in foliage (approximately 1% of the total Hg content) (St. Louis et al., 2001; Ericksen et al., 2003). Miller et al., (2005) estimated MeHg availability to terrestrial food webs using forest foliage and modeled deposition and concentrations of leaf, litterfall, precipitation (wet and dry), and particulate Hg in northeastern North America. Litterfall total Hg concentrations from these models were significantly correlated with the blood Hg levels of Bicknell's thrush (Rimmer et al., 2005). Conceivably MeHg in litterfall and contributions of foliar total Hg to saturated soils where potential methylation environments exist are providing an important basis for biomagnification of MeHg in invertebrates. Acidified environments further enhance methylation (Furutani and Rudd, 1980; Xun et al., 1987), and with the influence of heavy wet deposition of acid ions (i.e., acid rain), northeastern North America's landscape is generally more acidic than pre-industrial times (Driscoll et al., 2001). Soil acidification may impact bird populations in several ways (Graveland, 1998) including the depletion of soil calcium levels. Breeding birds have high demands of calcium for eggshell formation and proper juvenile growth. The widespread depletion of environmental calcium availability in northeastern North America is now linked to adverse effects on the distribution of wood thrush (*Hylocichla mustelina*) (Hames et al., 2002). Unfortunately, the strong link between environmental acidification with MeHg production and calcium depletion may be creating a scenario where their synergy has the potential for long-term, landscape-level impacts on insectivorous passerine populations across much of northeastern North America.

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Appendix 1. Basic geographic information, sampling time period, sample size by tissue and data source

Species	Latin Name	Project Geographic Area	Geographic Extent – Latitude		Geographic Extent – Longitude		Sampling Duration		Number of samples by tissue					
			Min	Max	Min	Max	Begin	End	Blood	Egg	Feather	Liver	Kidney	Muscle
Common Loon	*Gavia immer*	ME, MA, NH, NY, VT, NB, NS, ON, PE, PQ	42.350277	51.999444	−79.447777	−60.817	1986	2003	A: 770 J: 452	660	A: 631 J: 52	A: 30 J: 8	A: 10 J: 2	A: 18 J: 6
Black-crowned Night-Heron	*Nycticorax nycticorax*	ME, PQ	43.487777	48.460277	−74.577777	−68.53	1991	2003	J: 17				J: 6	
Great Blue Heron	*Ardea herodias*	ME, MA, PQ	45.030277	49.460277	−74.597222	−64.722222	1991	2003	A: 1 J: 17	2	J: 8		J: 8	
Canada Goose	*Branta bernicla*	ME, NB, NL, PE, PQ	46.133	57.283	−66.867	−54.167	1989	1999			A: 24	A: 7		A: 15
Wood Duck	*Aix sponsa*	ME, MA, NH, NB, PQ	42.338333	49.440555	−77.480277	−66.450	1990	2003	A: 13	16	A: 2	A: 2		A: 4
Mallard	*Anas platyrhynchos*	ME, NH,NY, NB, NL, NS, PQ	43.693888	52.917	−79.411944	−63.400	1990	2003	J: 3		A: 11 J: 1	A: 8		A: 16*
American Black Duck	*Anas rubripes*	NB, NL, NS, PE, PQ	43.783	54.167	−78.830	−53.250	1988	1993			A: 39	A: 39		A: 62*
Green-winged Teal	*Anas crecca*	NB, NL, PE, PQ	45.867	54.167	−78.838333	−53.500	1990	1992	A: 1		A: 8	A: 8		A: 24*
Ring-necked Duck	*Aythya collaris*	NH, NB, NL, PQ	45.500	51.994722	−79.280833	−55.083	1990	1997			A: 34 J: 1	A: 34 J: 1		A: 39*
Common Goldeneye	*Bucephala clangula*	ME, NB, NL, NS, PQ	43.633	53.167	−79.083888	−56.083	1989	2003	A: 6	22	A: 32	A: 32		A: 39
Hooded Merganser	*Lophodyte cucullatus*	ME, MA, NH, NB, NL, PQ	42.26	53.333	−79.024166	−57.833	1989	2003	A: 13 J: 5	45	A: 11 J: 1	A: 17		A: 20
Common Merganser	*Mergus merganser*	ME, NH, NY, VT, NB, NL, NS, PQ	43.5825	54.167	−79.182222	−53.500	1989	2003	A: 11 J: 69	26	A: 24 J: 32			A: 31
Osprey	*Pandion haliaetus*	ME, NH,PQ, NS, NB, NL, PE	42.968055	53.333	−78.752222	−60.433	1989	2003	A: 2 J: 58	23	A: 10 J: 62	A: 12 J: 11	A: 4 J: 9	A: 6 J: 1
Bald Eagle	*Haliaeetus leucocephalus*	ME, NH, NB, NS, PEI	43.836388	47.000	−71.611388	−59.920	1969	2003	J: 108	38	A: 9	A: 28 J: 2	A: 7 J: 2	A: 8 J: 2
American Woodcock	*Scolopax minor*	ME, MA	44.496666	44.496666	−69.237222	−69.237222	2000	2002	J: 4		J: 4			
Herring Gull	*Larus argentatus*	ME	44.76128	45.05178	−71.013383	−70.8182	1990	2003	A: 1 J: 15	8	A: 1 J: 1			

Appendix 1. Continued

Species	Latin Name	Project Geographic Area	Geographic Extent – Latitude		Geographic Extent – Longitude		Sampling Duration		Number of samples by tissue					
			Min	Max	Min	Max	Begin	End	Blood	Egg	Feather	Liver	Kidney	Muscle
Belted Kingfisher	*Ceryle alcyon*	ME, MA, NH, VT	42.172777	46.024166	−73.224166	−68.27444	1997	2003	A: 117 J: 183	16	A: 71 J: 13			
Downy Woodpecker	*Picoides pubescens*	MA	42.347350	42.347350	−71.382380	−71.382980	2003	2003	A: 1					
Willow Flycatcher	*Empidonax traillii*	MA	42.176240	42.390130	−71.382380	−71.319020	2003	2003	A: 1 J: 5					
Great Crested Flycatcher	*Myiarchus crinitus*	MA	42.28558	42.28558	−71.44901	−71.44901	2003	2003	J: 4					
Eastern Kingbird	*Tyrannus tyrannus*	MA	42.17386	42.364408	−71.31916	−71.37542	2003	2003		26				
Common Raven	*Corvus corax*	ME	44.97933	44.97933	−71.0215	−71.0215	2000	2000	J: 1					
Tree Swallow	*Tachycineta bicolor*	ME, MA	42.17325	44.94138	−71.5831	−70.9279	2000	2003	A: 53 J: 73	55				
Cliff Swallow	*Petrochelidon pyrrhonota*	ME	44.93642	45.15257	−71.0336	−70.4466	1999	2001	A: 19 U: 6					
Barn Swallow	*Hirundo rustica*	ME	44.97047	44.97047	−70.7166	−70.7166	1999	2001	A: 3					
Eastern Titmouse	*Baeolophus bicolor*	MA	42.347350	42.347350	−71.382380	−71.382380	2003	2003	J: 1					
Eastern Bluebird	*Sialia sialis*	MA	42.30914	42.38378	−71.49121	−71.38748	2003	2003	J: 7					
Bicknell's Thrush	*Catharus bicknelli*	ME, VT	45.183611	45.183611	−70.264722	−70.264722	1999	2003	A: 21		A: 18			
Gray Catbird	*Dumetella carolinensis*	MA	42.176240	42.390130	−71.382380	−71.319020	2003	2003	A: 2 J: 4					
Yellow Warbler	*Dendroica petechia*	MA	42.176240	42.390130	−71.382380	−71.319020	2003	2003	A: 4 J: 5					
Northern Waterthrush	*Seiurus noveboracensis*	MA	42.176240	42.347350	−71.382380	−71.319020	2003	2003	A: 2					
Common Yellowthroat	*Geothlypis thrichas*	MA	42.176240	42.390130	−71.382380	−71.319020	2003	2003	A: 4 J: 5					
Song Sparrow	*Melospiza Melodia*	ME, MA	42.176240	42.390130	−71.382380	−71.319020	2001	2003	A: 16 J: 18					
Swamp Sparrow	*Melospiza georgiana*	MA	42.176240	42.390130	−71.382380	−71.319020	2003	2003	A: 5 J: 13					
Red-winged Blackbird	*Agelaius phoeniceus*	MA	42.175555	42.351388	−71.381111	−71.321666	2003	2003	A: 3 J: 3					
Common Grackle	*Quiscalus quiscula*	MA	42.34631	42.35972	−71.37398	−71.36956	2003	2003		6				
Brown-headed Cowbird	*Molothrus ater*	MA	42.176240	42.176240	−71.319020	−71.319020	2003	2003	J: 1					
American Goldfinch	*Cardueli tristis s*	ME, MA	42.176240	43.731683	−71.382380	−70.566450	2000	2003	A: 12					

*(Mallard, Am. Black Duck, Am. Green-winged Teal, and Ring-necked Duck muscle tissues represent composites of adults and young-of-the-year).

Appendix 2. Tissue Hg levels (arithmetic mean ± SD and range) from nonlethal sampling efforts

Species	Blood (ww) Mean ± SD (Range)		Egg (ww) Mean ± SD (Range)	Feather (fw) Mean ± SD (Range)	
	Adult	Juvenile		Adult	Juvenile
common loon	2.04 ± 1.39 (0.05 – 8.63)	0.27 ± 0.34 (0.01 – 3.58)	0.78 ± 0.60 (0.01 – 9.00)	12.7 ± 6.6 (2.2 – 63.4)	5.4 ± 4.8 (0.3 – 25.7)
black-crowned night-heron		0.28 ± 0.13 (0.11 – 0.52)			
great blue heron		0.49 ± 0.56 (0.03 – 1.76)	0.09 ± 0.04 (0.05 – 0.12)		5.2 ± 2.0 (1.3 – 6.9)
Canada goose				0.3 ± 0.1 (0.3 – 0.8)	
wood duck	0.05 ± 0.04 (0.01 – 0.14)		0.12 ± 0.23 (0.01 – 0.94)	1.6 ± 0.3 (1.4 – 1.8)	
mallard		0.05 ± 0.03 (0.03 – 0.08)		0.9 ± 0.5 (0.3 – 1.8)	
American black duck				1.8 ± 1.2 (0.6 – 6.6)	
American green-winged teal				1.3 ± 1.4 (0.3 – 4.5)	
ring-necked duck				1.5 ± 0.8 (0.3 – 3.0)	
common goldeneye	0.21 ± 0.06 (0.15 – 0.31)		0.33 ± 0.18 (0.09 – 0.72)	2.8 ± 1.3 (0.8 – 7.0)	
common merganser	1.57 ± 0.59 (0.74 – 2.35)	0.60 ± 0.47 (0.03 – 2.29)	1.43 ± 0.86 (0.28 – 3.93)	8.0 ± 4.1 (3.3 – 17.7)	8.8 ± 5.4 (3.3 – 31.4)
hooded merganser	0.88 ± 0.55 (0.07 – 1.91)	0.68 ± 0.30 (0.34 – 1.13)	0.64 ± 0.44 (0.15 – 1.90)	10.4 ± 3.8 (2.7 – 18.0)	
osprey	1.42 ± 0.18 (1.29 – 1.54)	0.31 ± 0.20 (0.03 – 0.81)	0.19 ± 0.09 (0.06 – 0.38)	15.6 ± 13.6 (0.1 – 38.3)	8.2 ± 5.6 (0.1 – 26.5)
bald eagle		0.30 ± 0.27 (0.01 – 1.20)	0.45 ± 0.29 (0.03 – 1.29)	14.0 ± 6.8 (2.8 – 24.8)	
American woodcock		0.03 ± 0.01 (0.02 – 0.04)			0.2 ± 0.1 (0.1 – 0.3)
herring gull		0.46 ± 0.13 (0.28 – 0.72)	0.63 ± 0.55 (0.01 – 1.63)	18.1 ± 15.1 (4.4 – 57.0)	
belted kingfisher	0.99 ± 0.82 (0.07 – 4.57)	0.17 ± 0.18 (0.01 – 1.35)	0.56 ± 0.77 (0.03 – 3.03)	7.2 ± 7.6 (0.6 – 46.1)	8.0 ± 5.4 (3.8 – 19.8)
willow flycatcher		0.43 ± 0.25 (0.20 – 0.80)			
great crested flycatcher		0.09 ± 0.02 (0.07 – 0.11)			
eastern kingbird			0.12 ± 0.04 (0.04 – 0.21)		
tree swallow	0.41 ± 0.21 (0.11 – 1.00)	0.07 ± 0.03 (0.02 – 0.16)	0.19 ± 0.11 (0.04 – 0.64)		
cliff swallow	0.22 ± 0.10 (0.08 – 0.47)				
barn swallow	0.13 ± 0.03 (0.11 – 0.15)				
eastern bluebird		0.01 ± 0.01 (0.01 – 0.02)			
Bicknell's thrush	0.29 ± 0.26 (0.05 – 0.80)				
gray catbird	0.13 ± 0.07 (0.08 – 0.19)	0.05 ± 0.02 (0.03 – 0.07)			
yellow warbler	0.04 ± 0.03 (0.01 – 0.07)	0.04 ± 0.03 (0.01 – 0.08)			
northern waterthrush	0.92 ± 0.95 (0.25 – 1.59)				
common yellowthroat	0.28 ± 0.13 (0.15 – 0.44)	0.10 ± 0.05 (0.04 – 0.17)			
song sparrow	0.35 ± 0.30 (0.08 – 1.34)	0.21 ± 0.14 (0.01 – 0.56)			
swamp sparrow	0.74 ± 0.47 (0.22 – 1.45)	0.30 ± 0.14 (0.07 – 0.48)			
red-winged blackbird	0.67 ± 0.71 (0.20 – 1.49)	0.90 ± 0.38 (0.46 – 1.13)			
common grackle			0.04 ± 0.03 (0.01 – 0.07)		
American goldfinch	0.01 ± 0.01 (< 0.01 – 0.03)				

Appendix 3. Tissue Hg levels (arithmetic mean ± SD and range) from lethal sampling efforts

Species	Liver (ww) Mean ± SD (Range)	Kidney (ww) Mean ± SD (Range)	Muscle (ww) Mean ± SD (Range)
common loon - adult	29.7 ± 38.5 (1.9 – 154.0)	39.9 ± 25.1 (1.7 – 79.1)	4.10 ± 3.40 (0.40 – 15.90)
common loon - juvenile	14.9 ± 25.6 (0.3 – 92.6)	35.9 ± 24.9 (11.0 – 60.7)	0.90 ± 0.81 (0.20 – 2.40)
black-crowned night-heron		1.5 ± 0.3 (1.2 – 1.9)	
great blue heron		1.9 ± 0.9 (0.4 – 3.3)	
Canada goose	0.1 ± 0.3 (0.1 - 0.2)		0.04 ± 0.03 (0.02 – 0.14)
wood duck	0.3 ± < 0.1 (0.3 – 0.3)		
mallard	0.5 ± 0.2 (0.1 – 0.8)		0.13 ± 0.08 (0.02 - 0.26)
American black duck	0.6 ± 0.4 (0.1 – 2.0)		0.16 ± 0.12 (0.03 – 0.45)
American green-winged teal	0.8 ± 0.3 (0.2 – 1.3)		0.21 ± 0.15 (0.02 - 0.59)
ring-necked duck	0.6 ± 0.3 (0.2 – 1.5)		0.17 ± 0.11 (0.03 – 0.47)
common goldeneye	1.5 ± 1.8 (0.1 – 8.2)		0.33 ± 0.20 (0.05 – 0.88)
common merganser			1.71 ± 1.71 (0.08 – 6.77)
hooded merganser	4.7 ± 3.0 (1.1 – 12.2)		0.96 ± 0.35 (0.04 – 1.92)
osprey – adult	10.6 ± 11.2 (0.6 – 23.0)	15.7 ± 14.7 (2.3 – 36.7)	1.5 ± 1.5 (0.6 – 4.3)
osprey – juvenile	0.9 ± 0.4 (0.2 – 1.5)	1.1 ± 0.6 (0.3 - 2.1)	0.1
bald eagle – adult	2.2 ± 2.2 (0.6 – 11.8)	9.2 ± 12.4 (0.7 – 33.4)	0.4 ± 0.4 (0.2 – 1.5)
bald eagle – juvenile	1.2 ± 0.9 (0.6 – 1.9)	0.6 ± 0.5 (0.3 – 1.0)	0.3 ± 0.1 (0.2 – 0.3)
herring gull	3.6 ± 2.5 (1.0 – 8.2)		1.59 ± 1.32 (0.35 – 3.98)

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Embryotoxic Thresholds of Mercury: Estimates from Individual Mallard Eggs

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Abstract. Eighty pairs of mallards (*Anas platyrhynchos*) were fed an uncontaminated diet until each female had laid 15 eggs. After each female had laid her 15th egg, the pair was randomly assigned to a control diet or diets containing 5, 10, or 20 μg/g mercury as methylmercury until she had laid a second set of 15 eggs. There were 20 pairs in each group. After the second set of 15 eggs, the pair was returned to an uncontaminated diet, and the female was permitted to lay another 30 eggs. For those pairs fed the mercury diets, the even-numbered eggs were incubated and the odd-numbered eggs were saved for possible mercury analysis. Mercury in the even-numbered eggs was estimated as the average of what was in the neighboring odd-numbered eggs. Neurological signs of methylmercury poisoning were observed in ducklings that hatched from eggs containing as little as 2.3 μg/g estimated mercury on a wet-weight basis, and deformities were seen in embryos from eggs containing about 1 μg/g estimated mercury. Although embryo mortality was seen in eggs estimated to contain as little as 0.74 μg/g mercury, there were considerable differences in the sensitivity of mallard embryos, especially from different parents, with some embryos surviving as much as 30 or more μg/g mercury in the egg.

Mercury contamination of eggs has been suspected as a cause of impaired reproduction in wild birds (Barr 1986; Fimreite 1974), but associations between mercury levels in eggs and reproductive problems in field studies are complicated by the presence of other environmental stressors that may have an effect. Consequently, the results from controlled laboratory studies with mallards, black ducks (*Anas rubripes*), ring-necked pheasants (*Phasianus colchicus*), and chickens (*Gallus gallus*) have been used to help establish the concentrations of mercury in eggs that are associated with effects on avian reproduction (Heinz 1974, 1976, 1979; Finley and Stendell 1978; Fimreite 1971; Tejning 1967). The laboratory data have been used as a guideline for whether the mercury concentrations found in the eggs of wild birds might be harmful (Eisler 2000; Henny *et al.* 2000; Meyer *et al.* 1998; Scheuhammer *et al.* 2001; Thompson 1996; Wiemeyer *et al.* 1984; Wolfe *et al.* 1998).

In past laboratory studies with mallards, groups of breeding adults were fed different concentrations of methylmercury, and a mean mercury level was calculated from a sample of eggs from each group (Heinz 1974, 1976, 1979; Heinz and Hoffman 1998). When reproductive success of a group was significantly less than that of controls, the mean mercury level in eggs from that group was judged to be a harmful level. However, individual embryos likely differ in their sensitivity to methylmercury. As long as laboratory-generated findings with methylmercury continue to be used to help protect the reproductive success of wild birds, especially species that cannot tolerate much reproductive loss because their numbers are already low, it is important to know how sensitive individual embryos can be.

Our primary objective was to estimate the lowest concentrations of mercury in mallard eggs that would harm the most sensitive embryos. Our secondary objective was to determine how variable individual mallard embryos are in their sensitivity to methylmercury.

Materials and Methods

Care of Adults and Administration of Mercury Diets

Eighty breeding pairs of 1-year-old mallards (*Anas platyrhynchos*) (Kidder Game Farm, Milton, WI) were randomized to 1 m^2 outdoor breeding pens on April 15, where they had access to flowing water and a commercial game bird breeder diet (Purina Mills, St. Louis, MO). The diet contained about 20% crude protein, 2.5% crude fat, and 7% crude fiber. On April 24 we began collecting eggs from each pair. Eggs were labeled, stored in a Kuhl egg cooler (Flemington, NJ) at about 13–14°C, and at weekly intervals incubated in a Kuhl incubator at a temperature of 37.5°C and a relative humidity of about 54%. On day 25 of incubation we transferred the eggs to a Kuhl hatcher set at a temperature of 37.2°C and a relative humidity of about 70%.

Each of the 80 pairs was fed the untreated commercial diet until the female had laid 15 eggs. These first 15 eggs served to establish baseline data on the hatching success of each pairs' eggs prior to the start of mercury treatment. On the day a female laid her 15th egg, that pair was randomly assigned to one of the four treated diets: a control diet or diets containing 5, 10, or 20 μg/g mercury as methylmercury chloride. Methylmercury has been shown to be the main form of

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mercury found in the eggs of wild birds (Rumbold *et al.* 2001; Scheuhammer *et al.* 2001). Each treatment had 20 pairs of ducks. The dietary concentrations of mercury we used, especially the 10 and 20 μg/g treatments, were higher than had been used in some previous studies with mallards (Heinz 1974, 1976, 1979), but these concentrations were selected because we wanted mercury levels in eggs to increase greatly over a short period of time.

The methylmercury diets were prepared by first dissolving methylmercury in a small amount of acetone and then in a larger volume of corn oil. These solutions were then mixed with the breeder diet to make premixes, which were kept frozen until needed. The premixes were blended into a larger volume of breeder diet to make the final diets; these final diets contained about 0.2% corn oil. Two samples of each diet were analyzed to confirm the concentration of mercury. The samples of control diet were reported to contain less than 0.02 μg/g mercury. Recovery of mercury from the three treated diets averaged 89%. Mercury was analyzed by cold-vapor atomic absorption spectrophotometry at the Patuxent Analytical Control Facility, located at the Patuxent Wildlife Research Center.

For the 20 pairs selected to receive each of the three mercury-treated diets, only the first 14 eggs were incubated to establish a baseline for hatching success; the 15th egg was saved for possible mercury analysis. For the 20 pairs of controls, all of the first 15 eggs, plus all later eggs, were incubated. Eggs 16 through 30 were collected while the female was being fed her treated diet. On the day the 30th egg was laid, the pair was switched back to an untreated diet, and the female was allowed to lay another 30 eggs or until July 16, when the study was terminated. For those females switched to one of the three mercury-containing diets, we only incubated their even-numbered eggs and saved their odd-numbered eggs for possible mercury analysis.

Determining the concentrations of mercury that harmed individual eggs required some way of estimating how much mercury was in an egg while still allowing that egg to go through all the stages of incubation, hatching, and duckling survival. Our approach to estimating the mercury concentration in an incubated, even-numbered egg was to take the average of the mercury in the odd-numbered egg laid the day before and the odd-numbered egg laid the day after the even-numbered egg. During the period when an egg-laying female was on a constant methylmercury diet, mercury levels in eggs would be increasing; once the mercury diet was stopped mercury in eggs would decrease. In either case, the average of the mercury between the previous egg and subsequent egg should give a good estimate of what was in the unanalyzed egg.

We examined the fate of all the control eggs and all the even-numbered eggs of mercury-treated pairs. After eggs hatched, the ducklings were banded and kept for 6 days in heated pens provided with flowing water and untreated duck starter diet (Purina Mills). Because many hundreds of odd-numbered eggs were saved for potential mercury analysis, it was not feasible to have them all analyzed for mercury. Therefore, after the study of the hatching success of the even-numbered eggs and the 6-day survival of ducklings was completed, we carefully examined the fate of the eggs from each of the mercury-treated females. Some of the pairs had poor hatching success, even prior to being put on their mercury diets. We did not analyze many odd-numbered eggs from these poor-achieving pairs because failure of an egg to hatch could just as easily have been due to chance as to mercury poisoning. We limited most of our mercury analyses of odd-numbered eggs to situations where an even numbered egg (1) produced a deformed embryo, (2) produced a duckling that displayed neurological signs of methylmercury poisoning, or (3) failed to hatch when a series of eggs laid before it did hatch. In addition, with a few exceptions that are noted in the Results and Discussion, we did not include estimates of mercury in even-numbered eggs when more than 1 day separated the laying of the even-numbered egg from the laying of the neighboring odd-numbered eggs. Our estimates of mercury in these selected, even-numbered eggs were most likely to provide information related to true mercury toxicity, as contrasted to the normal amount of embryo mortality that even controls might be expected to suffer during incubation. Even with these restrictions, we analyzed over 200 odd-numbered eggs for mercury and related the estimates of mercury in even-numbered eggs to the toxic effects listed later.

Neurological Signs

The feeding of methylmercury to breeding mallards and black ducks has been shown to produce neurological signs related to characteristic brain lesions in some of their ducklings (Heinz and Locke 1976; Finley and Stendell 1978). Affected ducklings may appear normal when hatched, but within 1 to 3 days they begin to lose coordination and stagger about. In several studies, including the current one, we have never seen a control mallard duckling exhibit these neurological signs. Therefore, when ducklings from mercury-treated parents in the current study began to lose balance and stagger, this was an almost certain sign that they had been poisoned by methylmercury.

Deformities

Methylmercury has been reported to cause various kinds of deformities in mallard embryos, but deformities also occur in a small percentage of control ducklings (Heinz and Hoffman 1998; Hoffman and Moore 1979). In one large study with mallards, 6.1% of 1-week or older control embryos exhibited some form of deformity, primarily hydrocephaly (Heinz and Hoffman 1998). In the same study, 16.4% of the embryos from parents fed 10 μg/g mercury as methylmercury were deformed, many exhibiting deformities not seen in controls. In the current study, we could not be as certain that deformed embryos were caused by mercury as we were that neurological signs were the result of mercury in the egg.

Failure of Eggs to Hatch

Not all eggs, even controls, can be expected to hatch. A hatch of 70–80% of fertile control eggs is a good hatch for mallards (Gile and Meyers 1986; Heinz 1979; Heinz and Fitzgerald 1993; Heinz and Hoffman 1998). Therefore, to reduce the likelihood that the failure of an egg to hatch was unrelated to mercury treatment, we included data only for those pens that had a proven record of good hatching success prior to the switch to the mercury diets. After subtracting out the one or two unusable eggs that were occasionally cracked or infertile among the first 14 laid by the mercury-treated females, we defined good hatching success as there being no more than 1 unhatched egg out of the first 12, 13, or 14 usable eggs. Although not as certain an indicator of methylmercury poisoning as the exhibiting of neurological signs, the failure of an egg to hatch (when other uncontaminated or less contaminated eggs laid before or after this egg did hatch) provided information on threshold levels of mercury in mallard eggs that cause harm.

Mercury in Eggs That Hatched and from Which the Hatchlings Survived 6 Days

Although the main purpose of our study was to determine the lowest concentrations of mercury that harmed the most sensitive mallard embryos, we also were interested in how much embryos could differ in their sensitivity to mercury. One way to examine this variability was to determine how much mercury could be in an egg that did hatch and the duckling survived for the 6-day observation period. When an

embryo died or was deformed, we could not be absolutely certain that mercury was to blame. In contrast, we did know with absolute certainty when the estimated level of mercury in an egg did not kill an embryo, cause a deformity, or cause neurological signs in the hatchling. By examining the concentrations of mercury in eggs that produced healthy 6-day-old ducklings, we were able to understand how different mallard embryos can be in their sensitivity to methylmercury.

Results and Discussion

Neurological Signs

The lowest estimated mercury concentration in an egg that produced a duckling with neurological signs was 2.3 μg/g on a wet-weight basis and the highest value was 30 μg/g (Table 1). Given the specificity of this sign as something seen in previous studies only in mercury-poisoned ducklings, we believe the ducklings listed in Table 1 were poisoned by mercury and the estimated mercury levels in eggs produced these neurological problems. There were no control ducklings in this study that ever exhibited a loss of balance and staggering. Also, in the three groups that were switched to mercury-treated diets, there were no ducklings that exhibited neurological signs when they hatched from eggs laid prior to the start of the mercury diets. Most of the ducklings that exhibited signs of mercury poisoning hatched from eggs 22 through 30, which was during the period when mercury was in the diet of the mothers. Two ducklings that exhibited neurological signs hatched from eggs that were laid shortly after the cessation of the mercury diet.

Table 1. Estimated mercury concentration in eggs that produced ducklings that exhibited neurological signs of methylmercury poisoning

Concentration of Mercury in Parents' Diet (μg/g)	Female's Pen Number-Egg Number[a]	Mercury Estimated to Have Been in Egg (μg/g, wet weight)[b]
10	64-24	10
20	2-24	2.7
20	2-26	4.0
20	6-24	22
20	6-28	22
20	6-30	26
20	9-26	27
20	9-34	24
20	20-24	3.6
20	20-26	3.3
20	27-26	5.4
20	28-22	2.3
20	45-26	4.1
20	46-30	8.1
20	46-32	7.8
20	52-28	30
20	63-22	19
20	77-24	23

[a] The pen number of the female that laid the egg, followed by the egg number (according to the sequence in which the eggs were laid).
[b] The concentration of mercury in the egg producing this duckling was estimated by taking the average of the mercury in the egg laid the day before and the egg laid the day after this egg.

Deformities

The lowest estimated mercury concentration associated with a deformed embryo was 0.93 μg/g on a wet-weight basis and the highest value was 18 μg/g (Table 2). The estimate of 0.93 μg/g for egg number 62-48 (the 48th egg laid by female number 62) was based on the average between a value of 0.935 for egg 47 and 0.926 for egg 49. Egg 62-46 also produced a deformity and contained only 1.0 μg/g mercury, which was the average between values of 1.15 μg/g for egg 62-45 and 0.935 μg/g for egg 62-47. These two estimates seem to be very accurate averages based on mercury in the neighboring odd-numbered eggs. Female 62 also produced deformed embryos in her 20th egg, which contained 3.7 μg/g mercury, and in her 54th egg. The 54th egg is not listed in Table 2 because the odd-numbered neighboring eggs were not analyzed for mercury, but we know that the 54th egg almost certainly contained less mercury than the value of 0.926 μg/g reported for egg 49. The embryo from this 54th egg was badly deformed, with short legs, extra toes, a spoon-shaped upper bill, and an abnormal right wing. An unusual pattern of embryotoxic responses was seen in the eggs from female 62 in that, after egg number 20, which had a deformed embryo, eggs 22, 24, 26, and 30 (which would have contained more mercury than 22) all hatched. Egg number 28 was infertile. All of the even-numbered eggs from 32 through 44 also hatched, but they would have had more mercury than egg numbers 46, 48, and 54, which produced deformed embryos. We do not know why, but the embryos from this female seemed to be very different in their sensitivity to mercury.

Another female's egg (65-16) also produced a deformed embryo and contained an estimate of only 1.0 μg/g mercury. The 15th egg by female 65 was laid the day before the mercury treatment was started and was reported to contain 0.0278 μg/g mercury. The 17th egg was reported to contain 2.06 μg/g mercury. The estimate, based on eggs 15 and 17, could be biased toward the low side because the 15th egg was a pretreatment egg, and mercury could have jumped considerably by the 16th egg. Like female 62, female 65 also had an unusual pattern of embryotoxic effects in her eggs, suggesting differences in sensitivity among siblings. After egg 16, eggs 18, 20, and 22 all hatched. Thereafter, all of her eggs failed to hatch, either because they died (in two cases) or were infertile in all the rest.

In addition to the deformities listed in Table 2 or discussed so far, there were four additional embryos from mercury-treated parents that exhibited deformities, but either the neighboring eggs were not analyzed or the neighboring eggs were not laid within 1 day of the egg that produced the deformed embryo. One of these deformities, in the 32nd egg laid by a female fed 5 μg/g mercury was a deformed ball of tissue without any limbs. Only the 33rd egg from this female was analyzed and it contained 1.1 μg/g mercury; therefore, the deformed 32nd egg probably contained somewhat more than 1.1 μg/g mercury because it was laid one day closer to the time when the female was still being fed mercury.

A second additional embryo, from the 30th egg laid by a female fed 5 μg/g mercury, had a clubbed right foot that

Table 2. Estimated mercury concentration in eggs that produced embryos with deformities

Concentration of Mercury in Parents' Diet (μg/g)	Female's Pen Number-Egg Number[a]	Mercury Estimated to Have Been in Egg (μg/g, wet weight)[b]	Description of Deformity
5	16-26	3.8	Twisted toe on right foot; abnormal right wing; body generally underdeveloped
5	62-20	3.7	Elongated, spoon-shaped upper bill; legs and feet abnormal; abnormal joint on right wing
5	62-46	1.0	Spoon-shaped upper bill; hydrocephaly; short legs; extra toes; abnormal wings
5	62-48	0.93	Upper bill shorter than lower; short legs; extra toes; abnormal right wing
10	89-24	12	Lower bill longer; eyes poorly developed; stunted body; deformed skull
20	52-22	15	Left wing does not extend; foot deformed
20	65-16	1.0	One body sharing two fused heads; exencephaly; small eyes; deformed bill
20	77-22	18	Exencephaly; small right eye; lower bill longer and twisted

[a] The pen number of the female that laid the egg, followed by the egg number (according to the sequence in which the eggs were laid).
[b] The concentration of mercury in the egg with this deformed embryo was estimated by taking the average of the mercury in the egg laid the day before and the egg laid the day after this egg.

pointed backward plus rigid joints in its leg. It came from an egg that was estimated to contain 8.7 μg/g mercury, but the estimate was based on the average mercury in the 29th egg (10.1 μg/g), which was laid 2 days prior to the egg with the deformed embryo, and the 31st egg (7.29 μg/g), which was laid 1 day after. If anything, the 30th egg may have had slightly more mercury than the estimate.

A third embryo, from the 32nd egg laid by a female fed 10 μg/g mercury, had two heads partially fused together, a twisted upper bill, and a deformed right wing. Only the 33rd egg was analyzed for mercury, and it contained 12 μg/g mercury. Therefore, the deformed embryo came from an egg that probably contained somewhat more than 12 μg/g mercury.

A fourth embryo, from the 16th egg laid by a female fed 5 μg/g mercury, had a crossed bill and an indentation in the bill. The estimated value of only 0.44 μg/g mercury in this egg has to be viewed with caution for a number of reasons. First, deformities were limited to the bill. Second, the estimated mercury concentration of 0.44 was arrived at by taking the average of a value of 0.87 μg/g mercury measured in egg number 17 and a value of 0.01 for egg number 15. Egg 15 was laid while the female was still on its uncontaminated diet; it was reported to contain less than the detection limit of 0.0198 μg/g mercury, and consequently was assigned the value of 0.01, which is one-half the detection limit. Because of the uncertainty of how quickly mercury would build up in the very first egg laid after the switch to the mercury-containing diet, it is possible that the estimate of mercury in egg 16 was somewhat on the low side, but we can say with some assurance that this egg contained less than the 0.87 μg/g reported for the egg laid the following day. Finally, after the deformed embryo from egg number 16, eggs 18, 20, 22, 26, and 28 all hatched and eggs 24 and 30 died; therefore, this female also had an irregular history of embryotoxic effects.

A total of three embryos from the 20 breeding pairs of controls also were found to be deformed. Three deformed embryos is a low number considering that all but 4 of the 20 pairs of controls produced 60 eggs, and the lowest of these 4 still produced 48 eggs for incubation. One of the deformed controls had a slightly shorter lower bill than upper bill, another had a shorter upper bill plus exencephaly, and the third was a case of conjoined twins with exencephaly and a crossed bill.

Interpreting the results for deformities is clearly not as straightforward as for neurological signs. Though we do not rule out the possibility that as little as 1 μg/g or even less mercury did in fact cause the deformities we saw, at the same time we believe these estimates should be taken with some caution.

Failure of Eggs to Hatch

The lowest estimated mercury concentration in an egg that failed to hatch was 0.74 μg/g on a wet-weight basis and the highest value was 38 μg/g (Table 3). In addition to the mercury-treated pens listed in Table 3, 10 of the 20 control pens also met the criterion of having no more than one of their first 12–15 fertile eggs fail to hatch. Because the odd-numbered eggs from control pens were incubated along with the even-numbered eggs, there were as many as 15 eggs incubated from eggs 16 through 30. The mean hatching success of this second set of 15 eggs from the 10 control females was 89.2%, with a range from 80% to 100%. In only 1 of these 10 control pens did 2 eggs in a row fail to hatch. The uniformly high hatching success of eggs 16 through 30 for this select group of control pens suggests that it is unlikely that a series of eggs from the similarly select group of mercury-treated females in Table 3 would have failed to hatch by chance alone. Almost certainly, most of the eggs listed in Table 3 did die from mercury poisoning.

The lowest value in the table, 0.74 μg/g, came from pen 84, which hatched all 14 of its first set of eggs and all of the ducklings survived for 6 days. Based on this pretreatment success, we would have expected egg 16 to hatch unless

Table 3. Estimated mercury concentration in eggs that failed to hatch

Concentration of Mercury in Parents' Diet (μg/g)	Female's Pen Number-Egg Number[a]	Fraction of Fertile Eggs that Hatched During the Pretreatment Period[b]	Mercury Estimated to Have Been in Egg (μg/g, wet weight)[c]
5	11-28	13/14	5.2
5	34-26	13/14	7.1
10	82-28	13/14	14
10	82-30	13/14	17
10	89-24	13/14	12
10	89-26	13/14	13
10	89-28	13/14	14
20	19-34	14/14	3.4
20	46-34	14/14	13
20	63-20	11/12	13
20	63-26	11/12	29
20	63-28	11/12	30
20	63-30	11/12	38
20	65-24	12/13	23
20	84-16	14/14	0.74
20	84-26	14/14	33
20	84-28	14/14	34

[a] The pen number of the female that laid the egg, followed by the egg number (according to the sequence in which the eggs were laid).
[b] The numerator is the number of eggs that hatched, and the denominator is the number of fertile eggs that were incubated.
[c] The concentration of mercury in the egg that failed to hatch was estimated by taking the average of the mercury in the egg laid the day before and the egg laid the day after this egg.

mercury killed the embryo. The estimated mercury value for egg 84-16 was based on the average between the value of 0.009 μg/g for the 15th egg (mercury was below the detection limit of 0.0185 μg/g, and the egg was assigned a value of one-half the detection limit) and the value of 1.48 μg/g reported for the 17th egg. Even if the actual concentration of mercury in the 16th egg was closer to the 17th egg than to the 15th egg, it would still have been somewhat less than the 1.48 μg/g reported for egg number 17, and this would still be the lowest value in Table 3. What is peculiar about this pen is that eggs 18, 20, and 30 hatched, and they had estimated mercury values of 8.0, 22, and 31 μg/g. The embryos in eggs 22, 24, 26, and 28 all experienced some form of embryotoxic effect. Even-numbered eggs 32 through 52 all hatched, and only one duckling (84-32) failed to survive 6 days. The return to perfect hatching success after the cessation of mercury in the diet of female 84 suggests that the observed failures of eggs during the mercury treatment were in fact due to the mercury the female deposited in these eggs. However, it is still uncertain why some eggs laid after egg 16 but still within the period when the female was being fed mercury showed no indications of mercury poisoning.

If for some reason the estimated mercury level of 0.74 μg/g in egg 84-16 was not the cause of death of this embryo, then the next lowest value in Table 3 is the 3.4 μg/g value for egg 19–34. The fate of the eggs laid before and after egg 19–34 provide especially strong evidence that the embryo in egg 19–34 did die from mercury poisoning. Not only did all 14 eggs collected prior to the start of the mercury treatment for this female hatch, but egg numbers 16 through 28 (eggs laid while the female was on the mercury diet) all hatched. Egg 30, the last laid while on mercury treatment, failed to hatch, and its estimated concentration of mercury (based on an egg laid 3 days before and another laid 3 days after egg 30) was 4.8 μg/g. Egg 32 also failed to hatch; egg 31 was not analyzed, but egg 33 contained 4.2 μg/g mercury. Egg 32, being closer to the period when mercury was in the female's diet, should have contained slightly more than 4.2 μg/g. After egg 34, eggs 36 through 44 all hatched, presumably because mercury levels had declined to a safe level in these eggs that were more removed from the period when the female was fed mercury.

There were many other eggs from mercury-treated females that failed to hatch and for which we have estimates of mercury, but the data did not meet the rigorous criteria we imposed: (1) no more than one of the fertile pretreatment eggs failed to hatch and (2) the neighboring eggs analyzed for mercury had to have been laid no more than 1 day before and 1 day after the egg whose mercury concentration we wished to estimate. We did examine these data and there were no mercury values lower than even the second lowest value of 3.4 μg/g in Table 3.

Some of the estimated concentrations listed in Table 3 probably were higher than what was necessary to kill those embryos. For example, a series of increasing mercury levels is seen in Table 3 in eggs 63-20, 63-26, 63-28, and 63-30. In addition to these embryos that died, 63-16 and 63-18 also died, but we were only able to estimate that mercury was less than 5.0 μg/g (the level reported in egg 19). Egg 63-22 produced a duckling that exhibited neurological signs of methylmercury poisoning (this egg is listed in Table 1). Egg 63-24 hatched, but the chick was unable to walk normally and died. Egg 63-30, for example, probably did not require the 38 μg/g mercury it contained to kill the embryo; it probably would have died had its mercury level been closer to the value of 13 μg/g listed for egg 63-20. The reason 63-30 had 38 μg/g mercury was because it was laid later in the period when the female was being fed mercury; this egg, therefore, probably had more than enough mercury to kill the embryo.

Mercury in Eggs That Hatched and the Ducklings Survived

The results in Table 4, showing high levels of mercury that were not associated with harm, were not clear-cut. Except for pen number 84, there was not much difference between the mercury concentrations in eggs from the same female that were poisoned by mercury and those not poisoned. However, results discussed for data in the other tables suggest that less contaminated eggs may suffer from methylmercury poisoning, whereas much more contaminated eggs from the same female may hatch normally.

It addition to the differences in sensitivity that may exist among embryos from the same parents, it is clear that embryos from different parents can differ greatly in their sensitivity to methylmercury. As has been mentioned, the neurological results in Table 1 are the most reliable of all our data because we have never observed control mallard ducklings that exhibited these signs. Several ducklings hatched from eggs estimated to contain less than 5 μg/g mercury suffered neurological effects. For other ducklings, more than 20 μg/g mercury in the egg was needed to cause the same effects. The same observations can be made with the data in Tables 2 and 3; the concentration of mercury associated with embryotoxic effects can differ by more than an order of magnitude among the eggs from different parents.

Considerations of Mercury in Egg White Versus Egg Yolk

One final consideration in interpreting our results deserves mentioning. Because the egg-laying females in our study were in the process of building up mercury in their bodies during the period when mercury was fed and were losing mercury once they were returned to an uncontaminated diet, mercury in egg yolks and egg whites were rising and falling during these two periods.

In our study, when the female mallards were first started on their mercury-contaminated diets, the first eggs they laid would have had very little mercury in the yolk because the yolk, which takes many days to form, would have been almost completely formed prior to exposure to methylmercury. With these early eggs, an even greater than normal preponderance of the mercury would be in the albumen, which forms during the day the egg is laid. When chickens were fed methylmercury, about 95% of the mercury in their eggs was found in the albumen (Tejning 1967). During about the first half of incubation, an embryo relies almost completely on using the yolk for food (Freeman and Vince 1974). The relative contribution of mercury in the yolk and mercury in the albumen to the toxicity of mercury to the embryo is not known, but Tejning (1967) found that about 10 μg/g mercury in chicken eggs caused embryonic death before the 10th day of incubation, which is prior to the utilization of albumen as a food resource by the chicken embryo (Freeman and Vince, 1974).

Nearly all of the deformed embryos that appear in Table 2 were well beyond the stage when they were utilizing only yolk as a source of food, and nearly all of the embryos that failed to hatch and are listed in Table 3 were older embryos. Furthermore, most of the embryos that suffered toxic effects were not from eggs laid shortly after the switch to the mercury diets or shortly after the switch back to the uncontaminated diet. Consequently, we doubt that shifting ratios of mercury in yolk to albumen could have complicated our interpretations very much. However, with wild birds in nature, shifting ratios of mercury in yolk versus albumen could be common when breeding females move into and out of mercury-contaminated areas. Until the relative toxic contributions of mercury in the albumen versus the yolk are understood, it cannot be known whether basing toxic mercury thresholds on whole eggs could be misleading.

Conclusions

Our current study with individual eggs provides more reliable information than our earlier studies if one wishes to discover the lowest concentrations of mercury in eggs that will harm the most sensitive mallard embryos.

The findings for neurological signs, which were almost certainly related to mercury in the eggs, clearly showed that concentrations of mercury as methylmercury in excess of 2 μg/g on a wet-weight basis will harm sensitive mallard embryos. Data related to deformities and embryo mortality suggest that even lower concentrations of mercury in eggs may cause harm, but these findings were not as definitive as were the data for neurological signs. Therefore, we believe it is wisest to conclude that there is some evidence from deformities and mortality, although not conclusive, that wet-weight mercury concentrations of about 1 μg/g or perhaps a little below 1 μg/g can harm the most sensitive mallard embryos.

Our conclusions lend more reliability to the findings from other mercury studies in which thresholds for effects were expressed as mean concentrations of mercury in sample eggs from different treatment groups. When mallards were fed 3 μg/g mercury as methylmercury during two successive breeding seasons, toxic effects on embryos and young were associated with samples of eggs that contained between about 6 and 9 μg/g on a wet-weight basis (Heinz 1974, 1976). These older studies were different than the current study in that the breeding adults were started on their mercury diets well before the breeding season, and, consequently, mercury concentrations in eggs were fairly stable over the time when eggs were collected. Levels of mercury in eggs much below the 6–9 μg/g range were not produced in the early studies, so the mean levels of mercury in eggs were higher than what might have been minimal to cause harmful effects.

In another earlier study, breeding mallards were fed 0.5 μg/g mercury as methylmercury over three generations (Heinz 1979). When data for all three generations were combined, the number of 1-week-old ducklings produced per breeding pair was significantly lower for the pairs fed 0.5 μg/g mercury than for controls. Samples of 9–14 eggs were collected in each of the three generations for mercury analysis. The mean levels of mercury in eggs were 0.79, 0.86, and 0.84 μg/g on a wet-weight basis in the three generations, respectively. These three means from this 1979 study were the lowest to have been associated with reproductive impairment in mallards. Based on the findings of that study, and lacking mercury toxicity data specific to the wild birds they studied, some investigators doing

Table 4. Estimated mercury concentration in eggs that hatched and the ducklings survived 6 days

Concentration of Mercury in Parents' Diet (μg/g)	Female's Pen Number-Egg Number for Eggs that Hatched[a]	Mercury (μg/g, wet weight) Estimated to Have Been in Eggs That Hatched[b]	Fraction of Fertile Eggs That Hatched During Pretreatment Period[c]	Estimated Mercury in Other Even-Numbered Eggs That Suffered an Embryotoxic Effect and Were from the Same Female[d]
5	16-28	5.7	11/13	16-26 (3.8 μg/g), 16-30 (5.7 μg/g)
5	34-28	> 7.3	13/14	34-26 (7.1 μg/g)
	34-30	> 7.3		
10	89-30	> 14	13/14	89-24 (12 μg/g), 89-26 (13 μg/g), 89-28 (14 μg/g)
20	77-26	23	14/14	77-22 [deformed] (18 μg/g), 77-24 [neurological signs] (23 μg/g)
	77-28	23		
20	84-18	8.0	14/14	84-16 (0.74 μg/g), 84-22 [neurological signs] (≈28 μg/g), 84-24 [neurological signs] (≈29 μg/g)
	84-20	22		
	84-30	31		

[a] The pen number of the female that laid the egg, followed by the egg number (according to the sequence in which the eggs were laid).
[b] The concentration of mercury in the egg that hatched was estimated by taking the average of the mercury in the egg laid the day before and the egg laid the day after this egg.
[c] The numerator is the number of eggs that hatched, and the denominator is the number of fertile eggs that were incubated.
[d] These eggs were neighboring eggs laid by the same female that produced the eggs listed in the second column that hatched. These eggs failed to hatch or, if noted in brackets, the embryos were deformed or exhibited neurological signs of mercury poisoning. The estimated mercury concentrations are shown in parentheses (a ≈ symbol indicates that the estimate for the even-numbered egg used odd-numbered eggs that were not always only 1 day removed from the egg being estimated).

field studies have adopted a value of about 0.8 μg/g mercury on a wet-weight basis as a best estimate of how much mercury it might take to harm the reproductive success of the birds they studied (Henny *et al.* 2000; Lonzarich *et al.* 1992).

The fact that findings from our recent study, designed specifically to estimate the least amount of mercury that will harm an embryo, do not contradict the use of the 0.8 μg/g threshold, taken from Heinz (1979), raises an interesting question. Why did the earlier study, which was based on mean mercury levels in groups of eggs whose individual fate was not even determined, come up with about the same threshold as the more carefully designed current study? We believe the answer lies in the fact that the approximate value of 0.8 μg/g from Heinz (1979) was an average. The average probably included some mercury concentrations that were below an effect threshold *plus* some that were higher than what was needed to harm the most sensitive embryos. Even though the older study (Heinz 1979) yielded about the same answer, the findings from the current study, designed specifically to determine the lowest concentrations of mercury in mallard eggs that harm the most sensitive embryos, are more appropriate to establishing a toxic mercury threshold in eggs that can be used as a default threshold for the eggs of other species. It is important to recognize, however, that any default threshold assumes that the embryos of wild birds are of about the same sensitivity to mercury as are mallards. This may not be true. When results from various lab and wild birds were compared, there was evidence suggesting that reproductive success of some species may be more sensitive to methylmercury than it is for other species (Koster *et al.* 1996).

Our study was not designed specifically to determine what percentages of embryos or ducklings from eggs with varying amounts of mercury would be harmed, but we did gain some insights. The 60 pairs of mallards fed the three mercury diets was a large number, but if one were to use even larger numbers of breeding pairs it is possible that even more sensitive embryos could be discovered. However, from a practical point of view, we do not think that mercury levels in mallard eggs of 1 μg/g or less are likely to harm more than a small percentage of embryos. As our data in Table 4 showed, many embryos from eggs with 10 or more μg/g mercury seem to do well.

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Assessment of mercury emissions inventories for the Great Lakes states ☆

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Abstract

Anthropogenic mercury (Hg) air emissions for the eight Great Lakes states in 1999–2000 were evaluated by analyzing three inventories. The US Environmental Protection Agency (EPA) National Emissions Inventory (NEI) had the most complete coverage for all states, and total Hg emissions ranged from 4226 lb in Minnesota to 15,828 lb in Pennsylvania. Coal-fired electric utilities accounted for 52.7% of the region's Hg emissions, varying from 20.2% of the total in New York to 67.5% in Ohio. Other important contributors to regional emissions included municipal waste combustion (5.6%), mercury-cell chlor-alkali plants and hazardous-waste incinerators (4% each), stationary internal combustion engines (ICEs) (3.5%), industrial, commercial, and institutional (ICI) boilers (3.3%), and lime manufacturing (3.0%). Although medical waste incineration accounted for just over 1% of regional emissions using the original classifications, the inclusion of health care facilities that may have been inappropriately identified with other sectors would increase the sector to 4.5% of regional emissions (and decrease the stationary ICE sector to 1.4% of the regional total). There were substantial differences for some sectors between the NEI and the Great Lakes Regional Air Toxics Emissions Inventory (GLEI), as well as unexplained differences within inventories between states (particularly for the cement, lime, and asphalt industries, and for lamp breakage). Toxics Release Inventory data for 2000 mainly covered electric utilities, and differences from the NEI were significant for several states. An independent assessment indicates the possibility of underestimated Hg emissions by about twofold for ICI boilers, although data for the sector (in particular concerning fuel oil emissions) are highly uncertain. Limited data indicate the likelihood of significant underestimates of electric arc furnace mercury emissions in the NEI and GLEI inventories. Several measures are here identified for improving the reliability of the inventories, both for modeling of atmospheric transport and deposition modeling and for tracking progress in Hg reduction initiatives.

Keywords: Mercury; Emissions; Inventories; Great Lakes; Atmospheric deposition

1. Introduction

Mercury (Hg) contamination is widespread in the US Great Lakes region. Statewide consumption advisories due to Hg contamination covering at least one fish species are in place in seven of the eight Great Lakes states, covering inland lakes (Michigan, Minnesota, Wisconsin), rivers (Indiana), or both (Illinois, Ohio, Pennsylvania) (US EPA, 2003a). In addition to posing risks to human health (National Research Council, 2000), Hg contamination in the region also threatens certain fish-consuming wildlife, including loons and mink (Evers et al., 1998; Henry et al., 1998).

Atmospheric deposition has been recognized for some time as the major pathway by which most aquatic ecosystems are contaminated by ongoing Hg loadings (e.g., Jackson, 1997; Fitzgerald et al., 1998; Schroeder and Munthe, 1998; US EPA, 2000). For example, results from the Lake Michigan Mass Balance Study indicated that in the mid-1990s, 84% of total Hg loading to the lake (considering atmospheric and tributary inputs) was through atmospheric deposition (Landis and Keeler, 2002). Mercury is released to the atmosphere through both natural processes and human activities (Gustin,

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2003; US EPA, 1997a). Studies of inland lake sediment cores, however, show a significant anthropogenic Hg signal; the modern/preindustrial Hg flux ratios in eight rural Minnesota lakes ranged from 3.0 to 6.7 (Engstrom and Swain, 1997), and a similar range was found in the Adirondack region of New York (Lorey and Driscoll, 1999). A study of Great Lakes sediment cores showed significantly higher impact of human activity on Hg loadings to the lakes, with increases in areal loadings of up to 336-fold over preindustrial values in Lake Ontario. The increased loadings were attributed to more local industrial sources and distinct sediment mixing and integration processes (Pirrone et al., 1998). Although some of the inland lake data indicate that Hg loadings in the region peaked several decades ago, there is no indication that loadings have returned to preindustrial values, and anthropogenic Hg releases in the United States and other regions of the world are still significant (United Nations Environment Programme—Chemicals, 2002).

There have been increasing efforts in the past decade to develop more comprehensive Hg emissions inventories. Accurate inventory data—including information on parameters such as source location, stack height, and Hg emissions amount and speciation—are important as source terms for atmospheric transport and deposition models (e.g., Bullock and Brehme, 2002; Seigneur et al., 2003; Cohen et al., 2004). Accurate inventories are also necessary in the policy context, both in assisting in regulatory and voluntary program decision making, and in assessing progress in meeting broader policy goals, such as the virtual elimination targets for persistent toxic substances in the Great Lakes Water Quality Agreement and the Canada–US Binational Toxics Strategy (International Joint Commission, 1987; Environment Canada and US EPA, 1997).

Known historic Hg air emissions sources in the United States have included coal-fired electric utilities, certain mining practices, product- or use-related emissions (e.g., through waste disposal and certain manufacturing industries), and other incidental or use-related emissions. This article presents an overview and analysis of Hg emissions in the Great Lakes states in 1999, based on recently released inventories for the region. The analysis addresses most of the major Hg-emitting sectors; more detailed analysis of coal-fired electric utilities in the region will be presented in a separate article (in preparation).

2. Materials and methods

Three inventories covering broad activity sectors and including emissions data for Hg in the Great Lakes states were considered in this assessment. Brief overviews of the inventories and the approach used for data acquisition follow.

2.1. National Emissions Inventory (NEI)

The NEI is an effort by the US Environmental Protection Agency (EPA) to establish a comprehensive inventory for both criteria and hazardous air pollutants (HAPs). Intended uses of NEI data include in the National Air Toxics Assessment, in residual risk analysis, and in other atmospheric transport modeling efforts (Pope et al., 2002; ERG, Inc., 2003a). The first systematic national inventory for HAPs, including Hg, was compiled in 1990, through what was then termed the National Toxics Inventory (NTI), and a second NTI inventory for HAPs was completed for 1996. EPA released the Final Version 3.0 of the 1999 NEI in July 2003 (Pope et al., 2002; ERG, Inc., 2003a) .

The inventory for Hg and other HAPs is divided into point, nonpoint (NPS), and mobile sources. Point sources include major and area sources. Major sources, as defined in the 1990 US Clean Air Act (CAA), are those facilities that have the potential to emit $\geqslant 10$ tons per year of one HAP or $\geqslant 25$ tons per year of any combination of HAPs. Facilities whose annual emissions are below these thresholds are considered area sources. Data in the 1999 HAP inventory are derived from a number of sources, including state, local, and tribal agencies; industry; EPA data for regulated source categories, through Maximum Achievable Control Technology (MACT) standards; Toxics Release Inventory (TRI); estimated nonpoint data for sources not included in state, local, and tribal data; and 1996 NTI data for sources not otherwise included. For 1999 data, all Great Lakes states except Ohio had submitted point source emissions data to the US EPA (Pope et al., 2002; ERG, Inc., 2003a).

NPS emissions in the NEI consist of area sources with smaller and/or more diffuse emissions. In part, the definition refers to sources that the US EPA would regulate under provisions other than sections 112 or 129 of the CAA (Pope et al., 2002). Estimates for area sources are obtained using a 'top-down' approach, in which national-, regional-, and state-level emissions data are used to estimate emissions at the local (county) level. (Area sources where an individual emissions estimate can be obtained, but where the total emissions fall below the major category thresholds just mentioned, are considered point sources and are included in the point source NEI.) The hierarchy of data compilation involved state, local, and tribal data, supplemented by MACT NPS data, supplemented by EPA data based on emission factors and activity data. Though passed through quality assurance/quality control measures, differences in estimation approaches may lead to different estimates for the same category, limiting

between-state comparability. Among the Great Lakes states, four (Michigan, Minnesota, New York, and Wisconsin) submitted initial or revised NPS data to EPA (Pope et al., 2002; ERG, Inc., 2003b). For Ohio, emissions data for 15 HAPs, not including Hg, were submitted from the Regional Air Pollution Control Agency covering six counties in the southern part of the state (ERG, Inc., 2003a). In cases where no local, tribal, or state data are submitted, the EPA develops emissions estimates. Finally, no Hg emissions estimates were made for mobile sources in any jurisdiction in the 1999 NEI.

The data analysis approach for the NEI data was as follows. NEI point source and NPS state data files for 1999 (in MS Access format) for the eight Great Lakes states (Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin) were downloaded from the NEI website following finalization in summer 2003 (see US EPA, 2003b). Queries were developed and applied to each database file to identify only those facilities/units (point source data) or counties (NPS data) with reported Hg releases, based on the NEI pollutant codes for the nine forms of Hg contained in the 1999 NEI. Speciated Hg emissions (i.e., elemental gaseous Hg, gaseous divalent Hg—also known as reactive gaseous Hg—and particulate divalent Hg) were given only for coal- and coke-fired electric utilities in the 1999 NEI.

The approach for placing individual facilities in sectors was based on process characterization. Source Classification Codes (SCCs) and MACT code identifications were obtained from the NEI Lookup database available on the NEI website (US EPA, 2003b). SCCs were available for all records and were used initially to categorize all facilities. For cases where the SCC was nonspecific (e.g., 39999999, for miscellaneous manufacturing industries), MACT codes, if present, were used to assign facility categorization. Key Hg-emitting sectors with MACT codes were stationary internal combustion engines (ICEs) (0105); industrial/commercial/institutional (ICI) boilers (0107); stationary combustion turbines (0108) (which were considered with stationary ICEs in this analysis); integrated iron and steel manufacturing (0305); iron foundries (0308); steel foundries (0309); lime manufacturing (0408); Portland cement manufacturing (0410); taconite iron ore processing (0411); asphalt roofing and processing (0418); hazardous waste incineration (0801 (1–4)); municipal landfills (0802); chlorine production (1403) (limited to chlor-alkali plants in this analysis); medical waste incineration (1801); municipal waste combustion (1802); and electric utility boilers (1808 (1–3)).

A very small fraction of facilities had SCCs and MACT codes indicating two different sectors; these were predominantly in the stationary ICE and the ICI boiler categories (based on MACT codes). In these cases, facilities were placed in these two categories based on their respective MACT codes. For facilities with SCCs indicating ICI boiler or ICEs but a different MACT code (again, a small number of records, typically manufacturing sources with lower Hg emissions rather than any of the MACT categories just listed), the facilities were maintained in the boiler or ICE categories. Facilities having neither a specific SCC (e.g., 39999999 for miscellaneous sources) nor a MACT code were left in the miscellaneous category.

2.2. Great Lakes Air Toxics Emissions Inventory (GLEI)

The GLEI is a project of the eight Great Lakes states and Ontario to compile point, area, and mobile source emissions for toxic chemicals of concern in the region. A steering committee made up of representatives from each state and Ontario used a software tool and a standardized protocol to develop emissions estimates for each chemical. Emissions sources are organized by Standard Industry Classification (SIC) codes, SCCs, and Area and Mobile Source (AMS) codes. The most recent inventory, for 1999, consists of 213 HAPs, including Hg, and estimates for point, area, and mobile sources (GLC, 2003).

Although a standard protocol is in place, reporting restrictions and/or unavailability of data can lead to incomplete coverage of sources in each state. In addition, choice of emission factors for a specific source is made by individual state representatives based on information on the source (including control technologies in place) (O. Cabrera-Rivera, Wisconsin Department of Natural Resources, personal communication). For this analysis, Hg emissions data (aggregated by SIC codes and SCCs) for the Great Lakes states were obtained directly from the authors, because sector-specific Hg data were not yet available via the Internet (O. Cabrera-Rivera, personal communication). Because of the paucity of measured emission factor data for mobile sources (GLC, 2003), Hg emissions estimates for mobile sources were not considered in this analysis, although estimates were made in the 1999 GLEI.

2.3. Toxics Release Inventory

The TRI was established under the US Emergency Planning and Community Right-to-Know Act of 1986, with the first reporting year in 1987. The requirement originally applied to industrial facilities falling in SIC codes 20–39; having $\geqslant 10$ employees; and meeting a threshold requirement—either manufacturing or processing a chemical in excess of 25,000 lb/year, or otherwise using a chemical in excess of 10,000 lb/year (US EPA, 2001a). Reporting requirements have since been extended to other facilities, including federal facilities in 1994 and electric utilities, coal and metal mining

operations (with several exceptions), and other sectors for the 1998 reporting year. In 1999, EPA lowered reporting thresholds for Hg and other persistent, bioaccumulative toxic chemicals, applicable to the 2000 reporting year. The new requirement stipulated that any facility that manufactures, processes, or otherwise uses >10 lb of Hg or Hg compounds would be required to report (US EPA, 2001b).

The process involves industry submittal to EPA and state or tribal governments of chemical data (including amount and locations of chemicals stored on-site and estimated release amounts, with no requirement for measured values), compilation by EPA, and publication of annual inventories. Data reported include releases to air, discharges to water, land disposal, underwater injection, and on-site and off-site transfer (US EPA, 2001a). For this study, TRI air emissions data for Hg and Hg compounds on a state level for the Great Lakes states were downloaded via TRI Explorer (US EPA, 2003c) and processed in MS Excel. Because the lower reporting threshold did not take effect until the 2000 reporting year (and the large majority of Hg-emitting facilities did not meet the previous threshold requirements for Hg), this analysis focused on TRI data from the 2000 reporting year. (Although TRI data are incorporated into the NEI—unless overridden by other data—the two databases represent two different data collection efforts.) Because of the relatively small number of facilities reporting to TRI even with the lower threshold, Hg emission comparisons are made only for electric utilities and state totals.

2.4. Additional database for coal-fired utility hg emissions

This analysis also relied upon the 1999 Information Collection Request (ICR) for coal-fired electric utility steam-generating units, an effort to gather information on Hg emissions from the units in support of the US EPA regulatory determination for these units (US EPA, 2003d). Information derived from this effort included utility plant configurations and pollution control devices, coal Hg content, and estimated Hg emissions for all units in the United States. This has been the only systematic effort thus far to estimate, on the basis of measurements, Hg emissions from coal-fired utility units in the United States; data from this effort were incorporated directly into the 1999 NEI. For this study, plant and state totals for facilities in the Great Lakes states were obtained via the EPA Air Toxics website from the plant-by-plant mercury emission estimates file (US EPA, 2003d).

Because of its breadth of coverage, the US EPA NEI was the inventory examined in greatest detail in this assessment. In addition, more detailed comparisons between inventories were examined for several major sectors, and independent estimates of emissions were considered for two sectors.

3. Results

A compilation of Hg emissions estimates for the eight Great Lakes states from the US EPA NEI is given in Table 1. The data are presented in the format (i.e., point, nonpoint) used in the NEI database. A slightly different breakdown showing sector percentages for the Great Lakes states is shown in Fig. 1. Coal-fired electric utilities were responsible for 52.7% of the Hg emissions in the region in 1999, ranging in individual states from 20.2% in New York to 67.5% of the total in Ohio. This breakdown is quite different from 2000 estimates for Ontario, where the total fuel combustion sector was responsible for on the order of 20% of total Hg releases of 3028 kg (Trip et al., 2004; also see discussion in Hagreen and Lourie, 2004). The overall Hg emissions picture for the Great Lakes states evolved from earlier in the decade, at which point several other nonutility sectors were more prominent. The remainder of this section presents more detailed discussion of Hg emissions characteristics of several major sectors.

3.1. Electricity generation

Mercury exists naturally in coal and is released upon combustion. Electric utilities accounted for 85.6% of total coal consumption in the United States in 1999 (US DOE, 1999). Variables that influence the amount of Hg released from coal combustion include the coal rank and concentrations of other constituents (in particular chlorine), combustion conditions in the boiler, flue gas temperature and composition, fly ash properties, and pollutant controls after combustion (Kilgroe et al., 2002). Pollution control devices for other pollutants (i.e., sulfur dioxide, nitrogen oxides, or particulate matter, PM) can have an impact on Hg emissions from coal-fired units, although apart from fabric filters (for PM control), they are not generally effective at controlling elemental Hg (Kilgroe et al., 2002).

Total Hg emissions from coal-fired electric utilities in the eight Great Lakes states are given in Table 2 for four inventories. Although coal-fired utility boiler emissions data in the NEI were derived directly from the EPA ICR effort, Table 2 shows minor differences between the NEI and ICR databases for the eight states. More substantial differences in electric utility Hg emissions are seen in comparing 1999 ICR or NEI data to TRI data for 2000. These include ~30% and ~12% lower reported emissions in Illinois and Pennsylvania, respectively, and ~18% higher reported emissions in Minnesota and Ohio, compared with NEI data. Although the higher 2000 estimates for some states may be due to a

Table 1
Mercury emissions (lb) for Great Lakes States from US EPA NEI, 1999

Sectors	Illinois	Indiana	Michigan	Minnesota	New York	Ohio	Pennsylvania	Wisconsin	Regional total
Point sources									
Electricity generation									
Electric utilities—coal[a]	6016	4885	3094	1265	1028	7117	9961	2263	35,628
Other electricity generation	26	29	29	3	605	10	710	46	1458
ICI boilers									
Coal[b]	149	189	133	47	86	165	294	122	1187
Other	152	57	98	33	3	25	115	66	549
Stationary ICEs[c]	552	1344	195	39	138	0	118	0	2387
Chlor-alkali plants	—	—	—	—	—	1653	—	1082	2735
Cement manufacturing	1216	298	67	NA	40	38	58	9	1725
Lime manufacturing	1947	NA	NA	NA	NA	NA	42	13	2002
Petroleum refining	151	31	NA	NA	NA	189	318	4	692
Primary metal production	NA	NA	60	635	NA	NA	329	NA	1024
Secondary metal production	68	344	258	97	NA	0	NA	60	828
Incineration[d]	695	641	902	1413	1270	593	2289	371	8173
Other point sources[e]	1453	774	24	495	625	417	962	1091	5841
Nonpoint sources									
ICI boilers	38	32	52	14	132	90	72	34	464
Residential boilers	13	26	48	68	505	60	378	64	1161
Lamp breakage	82	30	69	27	463	62	49	29	811
Dental preparation and use	61	42	53	48	74	46	44	42	410
Laboratories	80	39	65	32	118	74	79	35	522
Other nonpoint sources	8	3	10	9	13	8	10	4	64
Total point sources	12,424	8592	4861	4029	3795	10,206	15,196	5127	64,229
Total nonpoint sources	282	172	297	197	1305	340	632	208	3433
Overall total	12,705	8764	5158	4226	5100	10,547	15,828	5335	67,662

Source: Data drawn from US EPA, 1999 NEI database (US EPA, 2003b), based on source categories (see text for details), with point/nonpoint source breakdown retained from EPA inventory.

NA, No data available in the database. Zero values indicate value <0.5 lb. Totals may not add exactly because of independent rounding.

[a] Includes only facilities with utility MACT code.

[b] Includes only facilities with SCCs indicating coal-fired boilers.

[c] Includes stationary combustion turbines.

[d] Detailed breakdown is presented in Table 4.

[e] As noted in text, may include facilities that could be considered to fall within specific categories above, but for which specific SCCs or MACT codes were lacking.

combination of increased reported emissions at individual units and TRI coverage of additional facilities not in the ICR database, the reasons for lower 2000 estimates for Illinois (in particular) and Pennsylvania are not clear.

Utility Hg emissions as reported through the GLEI varied quite considerably from those reported through the utility ICR, as shown in Table 2. For New York and Ohio, no utility Hg emissions estimates were obtained (on an SCC basis), and for Pennsylvania, utility Hg emissions were only 28.1% of the ICR totals (34.1% when based on SIC codes). Although agreement was better for four other states, the estimated utility Hg emissions total for Illinois was nearly double (SCC basis) the ICR total. Although some states have more limited ability to collect air toxics emissions data (GLC, 2003), the reasons for the considerable discrepancies in states where data are available are not clear.

3.2. *Industrial, commercial, and institutional boilers*

ICI boilers involve controlled flame combustion and provide thermal energy to run processes or machinery or to produce electricity. Process heaters are units in which combustion gases do not come into direct contact with process gases in the combustion chamber. These boilers and process heaters are used in a variety of ICI settings, including oil and gas extraction and petroleum refining, chemicals manufacturing, primary metals industries, national security operations, health services institutions, and educational institutions. These boilers can use coal, oil, natural gas, or other fuels as energy sources (US EPA, 2003e). Mercury is a trace constituent in the fuels (typically as mercuric sulfide in coal and as elemental Hg in fuel oil and natural gas) and can be released upon combustion. On the basis of an analysis of the emissions inventory database used in development of the MACT

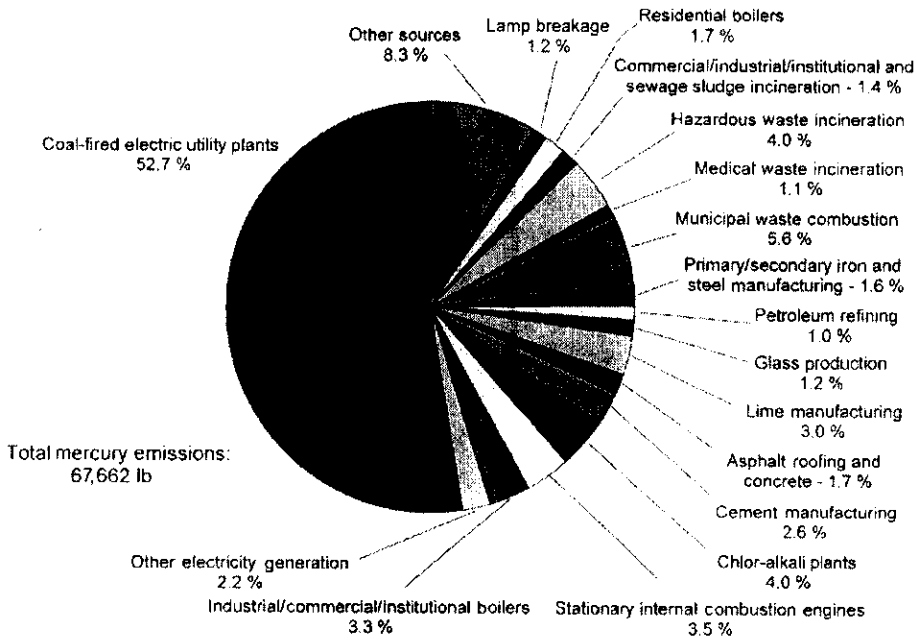

Fig. 1. Source category breakdown of combined 1999 Hg air emissions in the Great Lakes states. Data were compiled from US EPA 1999 NEI. (Total does not add to 100.0% as a result of independent rounding).

Table 2
Coal-fired electric utility Hg emissions (lb) for Great Lakes states from four inventories, 1999

Inventory	Illinois	Indiana	Michigan	Minnesota	New York	Ohio	Pennsylvania	Wisconsin
National Emissions Inventory[a]	6016	4885	3094	1265	1028	7117	9961	2263
Electric utility information collection request[b]	5989	4884	3083	1265	1027	7109	9959	2264
Toxics Release Inventory (2000)[c]	4169	5736	3010	1497	1175	8392	8780	2114
GLEI (SCC)[d]	11,868	4383	2612	1452	NA	NA	2800	2284
GLEI (SIC)[e]	12,006	4395	2662	1531	100	NA	3400	2577
2000 TRI % difference from ICR	−30.4	17.4	−2.3	18.4	14.4	18.0	−11.8	−6.6

[a] Data drawn from US EPA, 1999 NEI database (US EPA, 2003b), as described in text.

[b] From US EPA Air Toxics website (US EPA, 2003d), plant-by-plant mercury emission estimates: http://www.epa.gov/ttn/atw/combust/utiltox/stxstate2.pdf file.

[c] Year 2000 data, for Standard Industrial Classification (SIC) codes 4911, 4931, 4939, from US EPA TRI Explorer (US EPA, 2003c).

[d] 1999 Great Lakes Air Toxics Emissions Inventory, categorization based on SCC (data derived from spreadsheet provided by O. Cabrera-Rivera, personal communication).

[e] Same as note d, for categorization based on SIC codes 4911, 4931. Note that SIC codes 4911, 4931, 4939 are not exclusively for coal-fired electricity generation, but it is assumed that most Hg emissions are associated with coal-fired units.

standard for ICI boilers by the US EPA, ~42% of the inventoried ICI boilers and process heaters in the United States in the late 1990s were in the Great Lakes states (US EPA, 2003f).

Table 3 indicates Hg emissions estimates from ICI boilers in the Great Lakes states based on the NEI and GLEI. Data for the NEI show that for every state but New York, coal-fired ICI boilers were the largest subcategory of Hg emissions in the NEI database. The data also show that emissions from oil-fired boilers and boilers burning gas or other fuels were also relatively substantial in most states (as categorized in this assessment, the "other" category may include boilers burning a mix of coal and other fuels). Data from the GLEI show agreement within a factor of 2 for ICI Hg totals with data from the NEI database for four of the five Great Lakes states with available data. However, coal-fired emissions for Illinois boilers from the GLEI were eight times higher than the estimate derived from the NEI database.

The third set of data in Table 3 indicate Hg emissions estimates for ICI boilers based on statewide fuel

Table 3
ICI boiler Hg emissions (lb) for Great Lakes states from two inventories and fuel consumption approach, 1999

Inventory/sector	Illinois	Indiana	Michigan	Minnesota	New York	Ohio	Pennsylvania	Wisconsin	Regional total
NEI[a]									
Coal	160	194	134	48	98	169	301	129	1232
Oil	58	35	65	14	116	87	132	27	533
Gas and other	121	49	84	33	7	25	48	67	434
Total	339	278	283	94	222	280	481	223	2199
GLEI[b]									
Coal	1279	89	133	43	NA	NA	NA	220	1764
Oil	34	9	14	2	NA	NA	NA	8	66
Gas and other	83	42	57	33	NA	NA	NA	151	368
Total	1396	140	205	78	NA	NA	NA	379	2198
Estimate based on statewide fuel consumption									
Coal[c]	449	540	264	205	203	453	496	212	2822
Oil[d]	17	23	17	31	645	80	109	66	987
Total	466	563	281	236	849	533	604	278	3809

Overall totals may not add exactly due to independent rounding.

[a] Data drawn from US EPA NEI database (US EPA, 2003b), as described in text. Space heaters and boilers with SCCs not specifically indicating coal or oil (i.e., 102001xy–102005xy for industrial, and 103001xy and 103002xy for commercial/institutional) are included in gas and other category.

[b] 1999 Great Lakes Air Toxics Emissions Inventory data. Same category breakdown as for NEI (data derived from spreadsheet provided by O. Cabrera-Rivera, personal communication).

[c] Statewide coal consumption data for nonutility industrial plants are from DOE (1999) (Table 71, with estimate for New York based on 1998 and 1999 data for three states in that census region); emission factor of 5.43 lb per trillion BTUs from ERG, Inc. (2002) (Appendix A); heat content of coal from DOE (1999) (Table 107). Mercury emissions calculated as product of coal consumption data (converted to BTU basis from heat content data) and emission factors.

[d] Statewide oil sales data for industrial and commercial sectors are from DOE (2000), with adjusted distillate and residual fuel oil data from Tables 16, 17, respectively; average heating values for distillate and residual oils of 136,725 and 152,400 BTUs/gal, respectively, derived from US EPA (1997b) (Table 6–10, averaging No. 1, No. 2 fuel oil values (distillate) and No. 6 (residual) for central and eastern regions for each fuel); Hg emissions factors of 0.00887 and 8.80 lb per trillion BTUs, respectively, from ERG, Inc. (2002, Appendix A). Mercury emissions for distillate and residual fuels calculated as product of oil consumption data (converted to BTU basis from heat content data) and emission factors. Totals for both fuels are shown in table.

consumption data and emissions factors (derived for the US EPA MACT standard development process) for coal and fuel oil (see footnotes c and d in Table 3). In deriving these estimates, it was assumed that all such fuel in each category was consumed at ICI boilers in each state. For coal boilers, data from the fuel consumption approach are uniformly higher than NEI estimates for each state (ranging from 1.6- to 4.3-fold higher than the NEI estimates, for Pennsylvania and Minnesota, respectively), and higher for two of the five states with available data in the GLEI; the total regional emissions in the fuel consumption approach are 2.3-fold higher than the total estimate for all eight states in the more complete NEI. For oil-fired boilers, there are even greater discrepancies, with the fuel consumption estimate/inventory estimate ratio ranging from 0.3 (Illinois in the NEI) to 14.7 (Minnesota in the GLEI), and the fuel consumption approach overall leading to a nearly 2-fold higher estimate than the NEI for the eight states combined. Relative to other states, it is likely that the NEI estimate for New York oil-fired boiler emissions in particular is low; whereas the state's oil-fired ICI boiler Hg emissions in the NEI amounted to 22% of regional emissions for the sector in 1999, the state accounted for >65% of industrial and commercial sector residual oil sales in the eight states (US DOE, 2000).

Discrepancies in Hg emissions estimates for the three approaches could be due to a number of variables (including different coverage in number of boilers between each database), but one important issue is choice of emission factors. For coal combustion, the latest version of the US EPA Factor Information Retrieval Data System (FIRE 6.23) has Hg emission factors for bituminous/subbituminous-fired ICI boilers of 16 and 3.2 lb per trillion BTUs of heat input, for uncontrolled and controlled emissions, respectively (US EPA, 2003g; the latter converted based on average heating values in US EPA, 1997b). The average value derived by the US EPA in the ICI boiler MACT database (used as well in the fuel consumption approach presented in Table 3), which takes into account that some facilities have pollution control equipment installed, is 5.43 lb per trillion BTUs (ERG, Inc., 2002). This value is in the range of median values derived from the utility ICR for bituminous and subbituminous coals (7.1 and 5.0 lb Hg per trillion BTUs, respectively) (Kilgroe et al., 2002). If emission factors similar to the lower value from the FIRE

database (3.2 lb per trillion BTUs) were used in development of the NEI and GLEI on a considerable number of coal-fired ICI boilers in the region, this could partly explain the lower estimated Hg emissions in the inventories compared with the fuel consumption approach.

For petroleum products, the uncertainties surrounding emission factors are even higher than for coal. A US EPA compilation of estimated Hg concentrations in crude oils from various regions of the world gave mean values ranging from 0.1 to 1505 parts per billion (ppb), and mean Hg concentrations for gas condensates ranging from 15 to 3964 ppb (US EPA, 2001c). A review of petroleum-related Hg emissions indicated mean Hg levels in distillate and residual fuel oils ranging from 4 to 400 ppb (Wilhelm, 2001).

These wide-ranging values have been mirrored in US EPA emission factors derived over the past decade. Whereas a value of 7.0 lb Hg per trillion BTUs for residual and distillate fuel oil was used to estimate commercial and residential boiler Hg emissions in the US EPA Mercury Study Report to Congress, an emission factor of 0.6 lb Hg per trillion BTUs was derived from measurements on utility residual fuel oil (US EPA, 2001c). The latest version of the US EPA FIRE emission factor database has a distillate oil Hg emission factor (uncontrolled) of 3.0 lb per trillion BTUs of heat input, or intermediate between the two values above, while no value was available for residual fuel oils (US EPA, 2003g). As part of its ICI boiler MACT standard development, the US EPA derived emission factors for residual and distillate-fired boilers of 8.8 and 0.008871b Hg per trillion BTUs, respectively (ERG, Inc., 2002). The distillate factor is nearly 340 times lower than the distillate oil Hg emission factor in the FIRE database, whereas the residual factor is at the high end of published emission factors. These latter two factors were used to obtain the emissions estimates in the fuel consumption approach given in the last section of Table 3, and (given the emission factor differences) most of the Hg emissions were associated with residual oil. Assuming that the emissions factors from the ICI boiler MACT standard development are in fact more appropriate for the Great Lakes boiler population, the net effect (of a lower distillate factor but a high residual oil factor) may indicate underestimated total oil-fired ICI boiler Hg emissions in the NEI and GLEI inventories.

However, there is a crucial need to obtain more accurate data on Hg emissions from a wider variety of petroleum sources. A recent article reporting analyses of standard reference materials using a closed-system combustion technique with cold-vapor inductively coupled plasma mass spectrometry analysis noted the importance of low blanks in analyzing Hg concentrations at the low end of the scale in petroleum products. These authors found a substantially higher Hg level in residual fuel (3460 pg/g) than distillate fuel, but this value was still at the low end of other published data (Kelly et al., 2003). Converting to a heat content basis (following the approach of US EPA (2001c), such an Hg concentration would correspond to an emission factor of ~0.2 lb Hg per trillion BTUs, or over an order of magnitude lower than most emission factors most recently used by the US EPA. If this finding is more typical for residual oils, and if a typical distillate oil emission factor is indeed much lower than for residual oils—as found in ERG Inc. (2002)—Hg emissions associated with ICI oil-fired boilers in the Great Lakes region would be substantially less than indicated in the last row of Table 3 (assuming complete coverage of all such boilers). Such an assessment would obviously have to be confirmed with Hg concentration data on residual and distillate fuel oil known to be burned in a representative fraction of these boilers.

Finally, although the statewide fuel consumption approach yielded higher estimated total Hg emissions from ICI boilers than the two inventories examined, the regional estimate based on fuel consumption is still lower than what would be expected based on findings from the MACT regulatory development process (indicating ~26,000 lb for all fuels for the entire United States, with no Hg emissions from natural gas–fired boilers reported) (ERG, Inc., 2002). This is particularly puzzling given that the MACT regulatory development process reported a considerable number of ICI boilers in the Great Lakes region; for example, analysis of the inventory indicated that, on a boiler-per-segment basis (taking into account different fuels burned, but not boiler capacity), >55% of the coal-fired boilers were in the Great Lakes states (US EPA, 2003f). (Though not published in the literature, an assessment of regional boiler emissions in this database has been reported; Delta Institute, 2002.) The reasons for the lower than expected regional Hg emissions (or conversely the higher than expected national emissions from ICI boilers, considering that the fuel consumption approach yielded regional estimates within a factor of 2 of the NEI inventory estimate) are not clear. Issues to investigate would include emission factors, boiler population, capacity, and operational data.

An additional oil-related source of Hg emissions is petroleum refining. As shown in Table 1, estimated emissions varied considerably among the states, with no data available for Michigan, Minnesota, and New York, and highest emissions in Pennsylvania (318 lb). Much better information on the Hg content of crude oil, as well as ensuring complete coverage of all refining facilities, is necessary to better quantify Hg emissions from the sector.

3.3. Stationary ICEs and combustion turbines

Stationary ICEs range in size from 1 horsepower to >10,000 horsepower, and serve to generate mechanical or electrical power at fixed sites (US EPA, 2003h). Mercury can be present at trace levels in the fuels (e.g., gasoline, diesel oil, natural gas) and released upon combustion. Combustion turbines work similarly, by burning fuels (typically natural gas), and produce electricity, heat, or shaft power. Combustion turbines are used by the electric power industry, independent power producers, the gas pipeline industry, and chemical and industrial plants, with sizes ranging from 1 to ~200 MW (US EPA, 2003i). For purposes of this analysis, the two sectors were considered together.

Table 1 indicates that Hg emissions from stationary ICEs and combustion turbines ranged substantially, from <0.05 lb in Ohio to 1344 lb in Indiana, and the total for the eight states accounted for 3.5% of the region's Hg emissions. There was an equally wide range in the number of emissions records, from 8 in Ohio to 905 in Illinois. However, inspection of SIC, emission unit, and process codes indicated that 61.6% of the emissions for the sector in all states were attributable to health care facilities, mostly from emissions at facilities in Indiana (1333 lb) and Pennsylvania (118 lb). Considering the importance of these emissions, two possible explanations for these results are (1) mistaken placement of the facilities in the stationary ICE category rather than the MACT and SCC medical waste incineration category, or (2) if categorized appropriately as ICEs, erroneously high Hg emissions estimates. This is an issue that is easily resolved, and because of the magnitude of the emissions estimates and the likelihood of significantly differing Hg control requirements for ICEs as compared with existing requirements for medical waste incinerators (MWIs), it is obviously important for these facilities to be properly categorized. If these facilities were indeed misclassified, the stationary ICE Hg emissions would drop to 917 lb in 1999, or 1.4% of the regional total.

3.4. Waste incineration

In the mid-1990s, waste incineration accounted for 34% of the 158 tons of anthropogenic Hg emissions in the United States, according to the US EPA (1997a). Estimation of incinerator emissions in 1999 is particularly challenging, because it was a period of finalization and/or state implementation of federal emissions standards for municipal waste combustors (MWCs), MWIs, and hazardous waste incinerators (HWIs) (e.g., US EPA, 2000). Given this caveat, estimates for the Great Lakes states in 1999, derived from the NEI inventory, indicate that Hg emissions from incineration were almost certainly down from the mid-1990s, but were still appreciable, at 8174 lb, or 12.1% of the regional emissions total (see Table 4 and Fig. 1). MWCs were most prominent, at 5.6% of the regional total, followed by HWIs (4.0% of the total).

Table 4 also shows the significant variation in incinerator Hg emissions among states for a given sector. For example, Hg emissions from MWCs in the NEI ranged from none in Ohio to just over 1000 lb in Minnesota, New York, and Pennsylvania. Emissions from commercial and industrial solid-waste incinerators

Table 4
Mercury incinerator emissions (lb) for Great Lakes states from two inventories

Sectors	Illinois	Indiana	Michigan	Minnesota	New York	Ohio	Pennsylvania	Wisconsin	Total
NEI[a]									
Municipal waste combustion	28	24	536	1001	1047	NA	1002	132	3770
Medical waste incineration	51	244	50	2	38	17	283	36	721
Sewage sludge incineration	NA	NA	NA	379	3	81	14	NA	476
Hazardous waste incineration	128	372	316	32	182	494	990	194	2709
Commercial and industrial inclusive.	488	1	0	NA	NA	NA	NA	8	496
Total	695	641	902	1413	1270	593	2289	371	8174
GLEI[b]									
Municipal waste combustion	28	128	1295	329	316	NA	400	188	2684
Medical waste incineration	47	921	50	0	242	NA	NA	NA	1260
Sewage sludge incineration	0	118	162	405	3	NA	NA	NA	687
Hazardous waste incineration	NA	NA	NA	NA	NA	NA	NA	NA	NA
Commercial/instit./indust.	702	657	0	NA	348	NA	NA	NA	1707
Total	777	1824	1507	733	909	NA	400	188	6338

NA, Data not available in databases. Zero values indicated reported values <0.5 lb. Totals may not add exactly because of independent rounding.
[a] Data drawn from US EPA NEI database (US EPA, 2003b), as described in text.
[b] 1999 Great Lakes Air Toxics Emissions Inventory, categorization based on SCC (data derived from spreadsheet provided by O. Cabrera-Rivera, personal communication).

(CISWIs) were even more disparate; Illinois had 223 emissions records with a total of 488 lb Hg emissions, compared with a total of six records and 8.6 lb Hg for the other states combined. The differences may be due to a combination of differential category assignment, as well as potentially to more comprehensive reporting in Illinois. Comparison of Hg emissions estimates from incinerators in the NEI database to those in the GLEI show good agreement in some cases and others that vary considerably. State totals generally were within a factor of 2, for the five states with more complete GLEI data.

Some inconsistencies in reporting codes were also identified in the incineration sectors in the NEI. As noted previously in the discussion on stationary ICE emissions, 1470 lb of Hg emissions that may have been associated with MWIs were categorized as stationary ICE sources on the basis of MACT codes and/or SCCs. In addition, two health care facilities (one each in Illinois and Indiana, with emissions of 192 and 660 lb, respectively) were categorized in this analysis in the "other point source" category, because there were neither MACT codes nor SCCs for medical waste incineration. Including all of these facilities (amounting to 61 emission records) with questionable assignments in the medical waste category would increase the regional MWI Hg emissions contribution from 1.1% to 4.5% (i.e., to 3044 lb). In addition, the second largest incineration source of Hg emissions in Michigan—the Detroit Water and Sewerage District plant at 162 lb—had a MACT code placing it in the HWI category, rather than the sewage sludge category.

As a result of continuing implementation of incinerator regulations and closures of some facilities, it is probable that Hg emissions from the MWC, MWI, HWI, and CISWI sectors have decreased—possibly substantially in some cases—from the 1999 estimates shown in Table 4. But in light of the discussions above, substantial reductions would presumably not be expected at the facilities with conflicting classifications, if those in question have indeed been improperly classified as ICE rather than medical waste incineration units, given that MACT standards for ICE and combustion turbine units were just being finalized in 2003–2004 (US EPA, 2003h, i).

3.5. Manufacturing industries

As shown in Table 1 and Fig. 1, certain manufacturing processes can be significant sources of Hg emissions, but there are significant variations among states. Mercury-cell chlor-alkali plants are one of the largest Hg-consuming sectors in the United States, and there has been concern for some time about accounting for Hg use and releases at such plants, because of the considerable discrepancies between consumption data and reported release data (e.g., Ayres, 1997). The US Chlorine Institute, in partnership with the US EPA, developed a voluntary goal of reducing Hg use by 50% in the mercury-cell chlor-alkali sector by 2005, compared with a 1990–1995 baseline (Chlorine Institute, Inc., 2003). Annual Hg air emissions (as reported to the TRI (US EPA, 2003c) for the two mercury-cell chlor-alkali plants in the Great Lakes region, as well as the Hg use trend in the sector, are shown in Fig. 2 (Chlorine Institute, Inc., 2003). The data show the substantial decline in Hg consumption in the sector nationwide (86%) that has occurred since 1990; this decrease has been due in part to technology improvements in the cell room and plant closures (capacity was down 26% in that period) (Chlorine Institute, Inc., 2003). However,

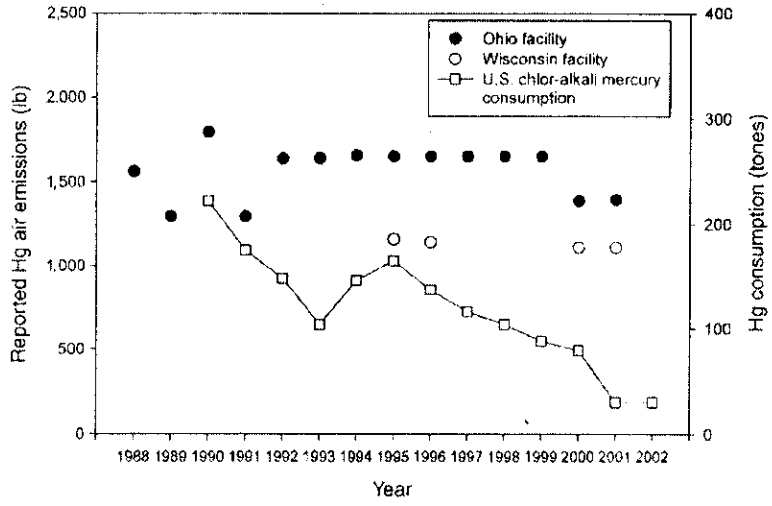

Fig. 2. Mercury emissions from chlor-alkali plants in Ohio and Wisconsin (TRI data; available through 2001), and Hg use in the US mercury-cell chlor-alkali industry (from Chlorine Institute, 2003).

despite the impressive reductions sectorwide in Hg consumption (which is presumably occurring to some extent at all plants), reported Hg air emissions have not changed as substantially at the two Great Lakes plants; when comparing the 4 years for which data are available at both plants, 2000–2001 average emissions were 15.8% and 3.6% lower compared with 1995–1996 average emissions, at the Ohio and Wisconsin plants, respectively.

Even with the reductions, at 30 tons/year, mercury-cell chlor-alkali plants remain one of the largest Hg-consuming sectors in the United States (Johnson, 2001). The US EPA recently finalized an Hg MACT standard for mercury-cell chlor-alkali plants that prohibits Hg emissions from new or reconstructed facilities, and establishes emissions limits for existing facilities (US EPA, 2003j). Although the US EPA anticipates continued Hg reductions from the affected facilities, the agency notes that a complete understanding of Hg mass balance in these plants—including the fate of most of the Hg consumed—remains to be achieved (US EPA, 2003j).

Another potential manufacturing source of Hg emissions is cement production. The United States was the world's third largest cement manufacturer in 1999 (van Oss, 1999). Mercury can be present in both the raw materials used and in the fossil fuels firing cement kilns, and emissions can occur during preheating of raw materials and thermal treatment in the kiln (US EPA, 1997b). (Hazardous waste–burning cement kilns are grouped under the hazardous waste category.) Estimated Hg emissions from cement manufacturing in the 1999 NEI database are given in Table 1. The data indicate that Illinois was responsible for the large majority (70.5%) of the region's Hg emissions for the sector. However, US Geological Survey (USGS) cement production data for the year indicate that Pennsylvania was the region's top producer (6690 thousand metric tons), followed by Michigan (5813), New York and Maine (data reported together, 3285), and then Illinois (2939 thousand metric tons) (van Oss, 1999). Cement production Hg emissions in the GLEI were substantially different from NEI values, with Indiana the top state at 308 lb and Illinois fifth in the region at 36.5 lb. Reasons for the differences are again not clear, but probably involve a combination of differences in number of emission records, capacity and operational information, and emission factors.

Two additional manufacturing sources of Hg emissions are lime manufacturing and asphalt production. At 1947 lb (97% of regional emissions), Illinois had the highest NEI Hg emissions associated with lime manufacturing for the region. Whereas the GLEI also listed Illinois as the largest source of lime manufacturing emissions, the value was 194.7 lb, or exactly 10.0% of the NEI total. USGS lime production data for 1999 indicate that the top-producing states in the Great Lakes region were Ohio and Pennsylvania (1870 and 1390 thousand metric tons, respectively), whereas Illinois, Indiana, and Missouri together (data were not available separately) produced a combined 3930 thousand metric tons (Miller, 1999). Mercury emissions from asphalt production (not shown) also differed significantly among the states, with Illinois accounting for 98.6% of the regional total of 1117 lb Hg emissions. The reasons for the disparate emissions in these manufacturing sectors are not clear, but possible explanations would include a disproportionate reliance on coal or coke in the firing process at facilities in Illinois, fewer Hg co-benefits in control devices in the state, overestimation of Illinois emissions (e.g., through use of higher emission factors), underreporting and/or underestimation of emissions in certain other states, or some combination of these variables.

3.6. *Primary and secondary metal production*

Primary and secondary production of certain metals can be important sources of Hg releases to the environment. One such sector is taconite (iron ore) mining and processing, in which taconite is mined and processed into iron oxide pellets for use mainly in steel production. In 1999, nearly all US crude iron ore production was concentrated at 10 operations in regions in two Great Lakes states: the Mesabi Iron Range in northeastern Minnesota and the Marquette Iron Range in the Upper Peninsula of Michigan (Kirk, 1999). This sector was the primary metal manufacturing sector with the highest Hg emissions in the Great Lakes region in 1999, with 471 lb of emissions in Minnesota according to the NEI. This was considerably lower than the value of 758 lb estimated by the Minnesota Pollution Control Agency (MPCA) for the state in 2000 (MPCA, 2002). However, four records in the NEI database with nonspecific SCCs (39999999) and no MACT code were in fact taconite processing facilities (with iron ore SIC codes), and including them leads to a total of 830 lb, much closer to the MPCA estimate for 2000. The two other US taconite processing plants are in Michigan (US EPA, 2002a). However, whereas the 1999 NEI had gas/oil-fired boiler emission records for these plants (with low Hg emissions), no other records for the facilities were identified.

Iron and steel manufacturing represent additional sectors of potential Hg emissions. US steel production can be divided into integrated mills and nonintegrated, or secondary, steel mills. Integrated producers use blast furnaces to smelt iron ore, which is then typically processed in basic oxygen furnaces to produce liquid steel. Secondary production involves 'minimills' and specialty mills, which use electric arc furnaces (EAFs) to melt lower cost (mostly scrap) metal, which is then

processed into products such as stainless, alloy-electrical, and tool steel (Fenton, 1999). (Though less commonly used in the industry now, due to increasing plant size in some cases, the term 'minimill' is retained here.) The major source of scrap metal entering US EAF mills is the ferrous fraction of scrapped automobiles. Because of the use of Hg in certain auto components (in particular lighting switches and antilock braking systems) and the large number of autos retired every year, potential mercury emissions from EAF steel production are substantial (Fenton, 2002; Maine DEP, 2003). EAF mills accounted for 46% (or 45.1 million metric tons) of US steel production in 1999 (Fenton, 1999).

Reported Hg emissions from iron and steel manufacturing facilities in the Great Lakes states for the NEI and GLEI inventories (data not shown) ranged from 0 and 3 lb in Wisconsin to 340 and 336 lb in Indiana, respectively (no data were available for New York and Ohio). Gray-iron foundries were responsible for 60.6% of total reported iron- and steel-related emissions in the NEI for the region (1099 lb), not counting taconite production (considered previously). However, missing from the two inventories are most of the EAFs in the region. For example, in 1999 there were 92 EAFs in the Great Lakes states (Anonymous, 2000); however, there were records in the NEI for only 13 EAFs, in place at either integrated iron and steel producers or iron foundries in the region. Though limited, data indicate that EAF minimills that process scrap autos can be important sources of Hg emissions; for example, annual emissions at a single Ohio facility were estimated at 660 lb (Sastry et al., 2002). (Though not in the peer-reviewed literature, this issue is addressed in detail in Ecology Center, 2001).

The reason for the discrepancy in coverage is that the US EPA delisted EAF operations at stainless and nonstainless steel manufacturing facilities from the MACT source list in 1996, reasoning that the facilities were not major sources of emissions (as defined in the Methods section) under the US CAA (US EPA, 1996). In addition, many iron and steel foundries are not major sources (US EPA, 2003k), and it appears that the majority of smaller plants are escaping notice in the inventories. In terms of US steel production, mills using the EAF technique are expected to account for >50% of production by 2010 (Crompton, 2001). Although US auto manufacturers had phased out Hg use in convenience lighting switches by the start of 2003 (Maine DEP, 2003), the large number of vehicles with such switches either retired or slated for retirement in the coming decade indicates a large pool of product Hg that may be released to the environment, but also for which a pollution prevention option (i.e., switch removal prior to shredding and metal reprocessing) is readily achievable.

3.7. Nonpoint sources

Data in Table 1 indicate that NPS Hg emissions were less than point source emissions in the NEI database, but not insignificant. (As noted previously, data for ICI boilers were considered with the point source data in the preceding discussion.) In contrast to the case with many of the point source sectors just examined, differences in NPS Hg emissions among states were generally less pronounced for most sectors. One exception was residential boilers, where both New York and Pennsylvania had considerably higher estimated emissions in the NEI (Table 1); this probably reflects, for the most part, increased reliance on fuel oil for home heating in those states (US DOE, 2000). Another exception was lamp breakage, where estimated Hg emissions for New York accounted for 57% of the regional total for the sector. Given that the state's 2000 population was only 23.3% of the regional total (US Census Bureau, 2001) and that estimated emissions from lamp breakage estimates would typically be made on a county population basis, the reason for the discrepancy is not clear. On the basis of apparent missing product-related Hg in Canadian inventories (Hagreen and Lourie, 2004), it is plausible that a similar situation might apply to certain area sources in the NEI and GLEI inventories for the Great Lakes states.

4. Discussion

4.1. Mercury emissions trends in the Great Lakes states

The US NEI has evolved from less complete earlier versions (Pope et al., 2002). Thus drawing solid conclusions on Hg emissions trends for specific sectors is difficult using data through 1999, as a result of changes in protocols and coverage; moreover, similar constraints apply to the GLEI (GLC, 2003). In addition, TRI data have been available only for 2 years at the lower reporting thresholds. Even though TRI data covered many of the top Hg-emitting sources beginning in 2000, the discrepancies in total emissions upon comparison to the 1999 NEI are considerable: 2000 state total TRI Hg emissions ranged from 27% (New York) to 113% (Ohio) of the 1999 NEI totals (data not shown).

In terms of national trends in Hg emissions, on the basis of an assessment before release of the final 1999 NEI data, US Hg emissions were estimated to have declined by >40% between 1990 and 2001, in particular as a result of a combination of use reductions and emissions control regulations (especially on incinerators) (Environment Canada and US EPA, 2002). (An analysis of final 1999 NEI data on a national basis in a recent report indicates that the national total emissions for

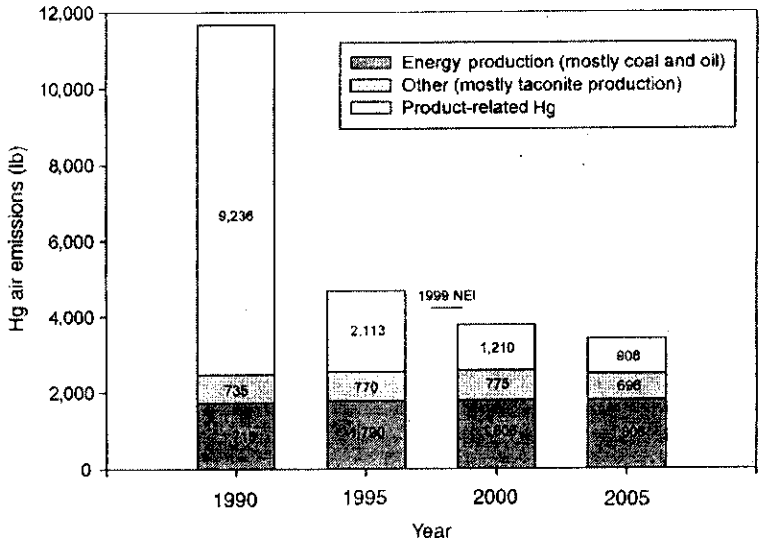

Fig. 3. Mercury air emissions trend in Minnesota for three broad sectors, with projection to 2005 (data replotted from MPCA, 2002).

1999 were consistent with this trend; see Northeast States for Coordinated Air Use Management, 2003).

One of the few internally consistent Hg emissions inventories in the Great Lakes region known to the authors is that developed by the MPCA for the state (MPCA, 2002). Estimated Hg emissions through 2000, and projections to 2005, are indicated in Fig. 3. Mercury emissions in the state were estimated to have dropped 68% from 1990 to 2000—a result attributed mainly by the agency to phase-out of Hg use in products (in particular batteries and paint), as well as emissions controls on incinerators (MPCA, 2002). (The 1999 estimated total emissions value from the NEI database derived in this analysis is indicated in Fig. 3 as well.) The 2002 assessment included a revised estimate (upward by 3000 lb) of paint-associated Hg emissions for 1990, reflecting the dynamic nature of Hg inventories (MPCA, 2002). The data show the impact on Hg emissions that product bans or voluntary restrictions can have, as well as the increased importance currently of incidental emissions through industrial sectors (such as metal mining and coal-fired electric utilities).

4.2. Policy implications and recommendations

Mercury emissions in the Great Lakes states amounted to 67,662 lb in 1999. On the basis of less complete 1996 NEI data (not shown and no longer available from the NEI website), as well as the more thorough analysis for Minnesota (Fig. 3), total anthropogenic Hg emissions in the Great Lakes region declined from 1996 to 1999. This was a period of implementation of emissions standards on incinerators that were anticipated to result in up to 95% reduction in Hg emissions (US EPA, 2000). The US EPA has finalized or is in the process of finalizing air pollution control rules that will affect a number of Hg-emitting sectors in the region, including iron and steel foundries, taconite plants, chlor-alkali facilities, and ICI boilers (US EPA, 2003l). In addition, the agency recently proposed several rule options covering coal-fired electric utilities (US EPA, 2004). Implementation of these rules will influence the magnitude and makeup of Hg emissions in the region and elsewhere in the United States for the next decade or more.

This analysis has shown that despite improvements in emissions inventories—including coverage of more facilities and availability of increased measurement data (e.g., for coal-fired electric utilities)—questions remain about the coverage and accuracy of Hg air emissions estimates in the Great Lakes states. Improvements in the Hg inventories are warranted in a number of areas, including increasing measurement efforts on diverse sources to improve emissions factors and permit increased use of actual stack test data, ensuring proper categorization based on source type (in particular in submissions to the NEI), improving GLEI coverage in states with limited data, and increasing coverage of area sources. There is a clear need for better data on Hg emissions from certain sectors in particular, including mercury-cell chlor-alkali facilities, EAFs, and ICI boilers (in particular those burning fuel oils). In addition, a better understanding of the range of Hg levels in petroleum products will assist in bounding mobile source emissions, which, though estimated (based on detection limits) in the GLEI, were not included in the 1999 NEI because of a lack of data.

One challenge in making improvements to the process is likely to be the disparate personnel and offices involved in inventory development, which in some cases

may amount to at least four different parties involved (for the three principal inventories considered here and the US EPA Emission Factors and Inventory Group). An additional challenge is the absence of reporting requirements: the US EPA announced in 2002 that Hg and other hazardous air pollutants would not, for the time being, be subject to mandatory emissions reporting requirements (US EPA, 2002b). Without such requirements, developing a comprehensive Hg emissions inventory that can more accurately show emissions trends for different sectors—as is currently done annually for criteria pollutants (US EPA, 2003m)—will be much more challenging. Having good inventories is essential both for understanding the transport and fate of Hg in the environment, as well as for gauging progress toward policy goals for Hg and other chemicals of concern in the Great Lakes system and beyond.

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EFFECTS OF WATERBORNE MERCURY ON TERRESTRIAL WILDLIFE AT CLEAR LAKE: EVALUATION AND TESTING OF A PREDICTIVE MODEL

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Abstract—Birds and mammals exposed to waterborne mercury (Hg) and methylmercury (MeHg) were collected and/or sampled at Clear Lake, California, USA, to field test the predictive wildlife criteria model developed for the Great Lakes Water Quality Initiative (GLWQI). Tissue samples collected from sampled animals were analyzed for Hg and organochlorine residues, and for selected physiologic parameters known to be affected by Hg. All mammalian organ tissues analyzed contained less than 12 ppm total Hg, wet weight. All avian tissue samples analyzed contained less than 3 ppm total Hg, wet weight. No evidence of Hg-associated health effects was found. Tissue Hg residues were compared with water, sediment, and animal food samples to characterize bioaccumulation of mercury in the Clear Lake food web. Total Hg bioaccumulation factors for the Clear Lake site closest to the Hg source were: TL-2: 11,100; TL-3: 31,200; TL-4, 190,000. Our results support the final wildlife criterion (1,300 pg/L) and suggest that the GLWQI model, with site-specific modifications, is predictive for other Hg-bearing aquatic systems.

Keywords—Wildlife Methylmercury Great blue herons Trophic transfer

INTRODUCTION

The United States Environmental Protection Agency (U.S. EPA) Office of Water has undertaken investigations to determine toxicity thresholds of waterborne contaminants for terrestrial wildlife. Upper trophic level piscivores/omnivores, such as great blue herons, mink, and raccoons, are at risk from environmental contaminants that bioaccumulate in aquatic foods chains. Blackbirds and swallows, which feed on emergent aquatic insects, also may be exposed to waterborne contaminants. This study reports the sampling of fish-eating birds and mammals and insectivorous birds from Clear Lake, California, USA, which is presently contaminated with mercury (Hg) and methylmercury (MeHg), and was historically contaminated with organochlorines (OCs) [1–4]. Unless otherwise noted, Hg refers to total Hg.

The goals of this project were to test the assumptions and methods of the Great Lakes Water Quality Initiative (GLWQI) model by comparing the wildlife criteria derived from the model with measurements in a Hg-bearing system other than the Great Lakes, and to suggest modifications to improve the model's predictive power. The model was developed for estimating the threshold concentrations of water borne contaminants that constitute a health threat to vertebrate terrestrial wildlife [5–7]. Water quality criteria previously published by the U.S. EPA were based on tests on aquatic organisms and laboratory animals and were designed to protect humans, fish, and aquatic invertebrates [8]. Because of distinct physiologic characteristics and different patterns of exposure, water quality criteria developed for aquatic organisms and humans may not be adequately protective of terrestrial wildlife. Therefore, the GLWQI Committee, under the sponsorship of the U.S. EPA's Office of Water, organized an effort to derive water quality criteria for wildlife, or wildlife values (WVs).

Efforts to establish water quality criteria specifically for wildlife originally followed the general model first employed the Wisconsin Department of Natural Resources and refined by Peterson and Nebeker [9] and the GLWQI [10]. After review and revision, the model adopted for the final rulemaking was [5–7,11,12]

$$WV = \frac{\dfrac{TD}{UF_A + UF_S + UF_L} + Wt}{W + \sum (F_{TLi} + BAF_{TLi})}$$

where WV is the wildlife value (mg/L); TD is the test dose (mg/kg/d) for the test species, either a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL); UF_A is the uncertainty factor (UF) for interspecies extrapolation; UF_S is the uncertainty factor for subchronic to chronic exposure extrapolation; UF_L is the uncertainty factor for LOAEL to NOAEL extrapolation; Wt is the average weight in kilograms (kg) of the representative species; W is the average daily volume of water (L/d) consumed by the representative species; F_{TLi} is the average food consumption (kg/d) at trophic level i by the representative species; and BAF_{TLi} is the bioaccumulation factor at trophic level i (i.e., the ratio of the toxicant concentration in wildlife food to its concentration in water; the units of the BAF are L/kg). Given the prominence of Hg and MeHg as contaminants of concern to wildlife in the Great Lakes, and because of their propensity to bioaccumulate in food chains, these contaminants were singled out for special consideration, via a field validation of the method used to derive the WV. Clear Lake was chosen as the site for the field validation study because it is contaminated with Hg and MeHg, and because it is home to species different from those used to derive the GLWQI model, but that also occupy the upper trophic level piscivore niche. Clear Lake therefore

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provided an opportunity to test the rigor and transportability of the model.

Peterson and Nebeker [9] identified three information deficits bearing on hazard assessment for wildlife: toxicity of environmental contaminants to wildlife, the dynamics of toxicant accumulation in ecosystems, and the relative importance of various routes of toxicant exposure to wildlife. Therefore, our objectives were to compare the water Hg concentration at Clear Lake with the toxicity threshold predicted by the GLWQI model, to compare tissue Hg concentrations of Clear Lake wildlife to tissue Hg concentrations associated with published NOAELs and LOAELs, to compare dietary Hg intake of Clear Lake wildlife with the dietary threshold concentration assumed by the GLWQI criterion, and to determine whether this level of Hg exposure affects the health and breeding success of Clear Lake wildlife. In addition, we compared Hg residues in tissues and samples obtained nonlethally with Hg residues in tissues of toxicologic significance to evaluate the suitability of nonlethal sampling as a substitute for traditional tissue analysis.

The first task of the project was to use data derived from controlled feeding studies in which the contaminant is administered to the test animals in food or water to estimate the NOAEL. This NOAEL value, and information on exposure to the contaminant in nature, were used to estimate the waterborne concentration causing exposure equivalent to the experimentally derived NOAEL [13]. Using this method, the GLWQI Committee derived a WV of 1,300 pg/L in the final GLWQI rulemaking [5]. The second part of the project, reported here, was to evaluate the model's predictive strength by field testing it with free-living birds and mammals in an Hg-bearing aquatic system, under actual exposure conditions. By comparing growth, reproductive success, and other indicators of wildlife health between Clear Lake animals and animals unaffected by Hg, we hoped to further estimate the impact of a known water concentration of Hg, and compare that concentration with the WV concentration determined by the model calculations.

From previous and concurrent work, we knew that Hg concentrations at Clear Lake were low relative to other Hg-bearing systems for which descriptions have been published. [14–19] Therefore, we had reason to anticipate small, sublethal effects. For this reason, and because we were limited to small sample sizes for each species surveyed, we tried to maximize the number of parameters assessed, including as many measures of population and individual animal's health as could be achieved within the time and budget resources available. Data presented here include tissue Hg concentrations of animals collected or sampled at Clear Lake, growth, measures of reproductive success, and histologic and hematologic analyses. These findings were used to critique the GLWQI WV model in its draft form, and to suggest improvements to the model. Ideally, we would also have sampled the species of birds and mammals that were used to derive the final WV, that is, bald eagle, herring gull, kingfisher, and otter, as well as mink. All these occur at Clear Lake, either seasonally or year-round. In practice, species selection criteria were availability, budget, and logistical and regulatory access.

Clear Lake and Sulphur Bank Mercury Mine

Clear Lake was chosen as a field-testing site for the GLWQI wildlife toxicity model because of the abundance and diversity of terrestrial wildlife there potentially exposed to waterborne Hg, and because of the concurrent study being conducted on sediment, water, and lower trophic level organisms by staff from the University of California at Davis under the sponsorship of U.S. EPA Region 9 Superfund [14,20,21]. Clear Lake is California's largest natural lake. It lies between the Inner and Outer Coast Ranges, an area that contains cinnabar-bearing serpentine soils. Sulphur Bank Mercury Mine (SBMM) is located on the Oaks Arm of Clear Lake (Fig. 1). During past open-pit cinnabar mining and ore processing activities, Hg-containing wastes were discarded in Clear Lake. Erosion of tailing piles and leaching from the open pit (now filled with water and referred to as Herman Impoundment) continue to deposit Hg to already contaminated sediments. Inorganic Hg is converted to MeHg in Clear Lake sediments by microbial action. Methylmercury is the contaminant of primary concern from SBMM, because of its greater rate of absorption (98%) compared to inorganic Hg (1%) [22], and because MeHg bioaccumulates and biomagnifies at higher trophic levels. Methylmercury concentrations in excess of U.S. Food and Drug Administration guidelines have been found in Clear Lake fish, and have prompted the California Department of Health Services to issue a public health advisory recommending a limit on the consumption of Clear Lake fish, which has remained in effect since 1986 [20]. Sulphur Bank Mercury Mine became a Superfund site in 1990. Remediation work is currently under way, under the direction of U.S. EPA Region 9.

Historically, Clear Lake was also contaminated with OC compounds, mostly as a result of a midge control program in which dichlorodiphenyldichloroethane (DDD) was deposited directly into the lake. The bioconcentration of dichlorodiphenyltrichloroethane (DDT)/DDD in western grebes was reported by Herman and coworkers shortly thereafter [23]. Because of this potentially confounding source of contaminant impact on wildlife, we also analyzed a subset of sampled species for OC residue.

Mercury concentrations at SBMM, Clear Lake water, sediment, and lower trophic level biota have been sampled in the course of a concurrent study conducted by members of the Institute of Ecology, University of California, Davis, under contract to U.S. EPA Region 9 Superfund. Suchanek and coworkers [24] measured total Hg and MeHg water concentrations monthly at six sampling sites in Clear Lake from 1992 through 1996, collecting samples from both surficial and deep water layers, six to seven samples per site (concentrations are yearly averages). Total sediment Hg in Oaks Arm, the site closest to the mine, was 40 to 370 ppm with an average of 220 ppm (μg/g); total sediment Hg was 0 to 50 ppm at other sites sampled throughout the lake. Oaks Arm total Hg was 81 ng/L (ppt) in unfiltered deep water and 22 ng/L in unfiltered surficial water. Unfiltered surficial water generally contains less than 100 ppt at all sampling sites. Corresponding MeHg measurements for Oaks Arm were 10 ppb (ng/g) in sediment, and 0.1 to 1 ppt (ng/L) in unfiltered deep water. Unfiltered deep water MeHg is generally between 0.01 and 0.4 ppt elsewhere in the lake. Methylmercury concentrations in Clear Lake water are therefore close to the limits of detection of the most sensitive analytical method, 0.05 ppt (ng/L) by cold-vapor atomic fluorescence spectroscopy [25–27].

Great blue herons (*Ardea herodias*), double-crested cormorants (*Phalacrocorax auritus*), raccoons (*Procyon lotor*), mink (*Mustela vison*), and river otter (*Lutra canadensis*) are among the upper trophic level piscivores at risk from environmental contaminants that bioconcentrate in aquatic foods chains. Three large heron colonies were located on the shore

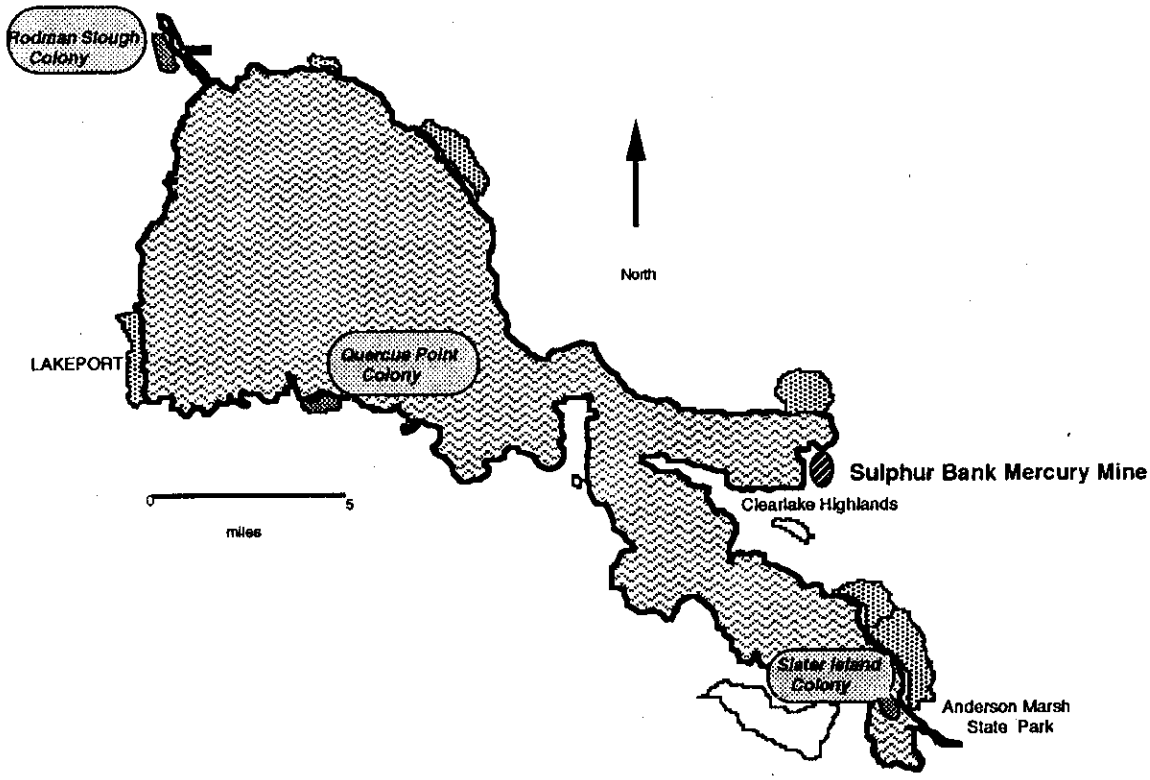

Fig. 1. Map of Clear Lake, California, USA, showing Sulphur Bank Mercury Mine (SBMM) and great blue heron colonies.

of Clear Lake in 1993, and two in 1994. Double-crested cormorants also nested at one of the three sites (Fig. 1). Great blue herons are widely distributed and often nest near contaminated sites, so a substantial fund of comparative data is available [28–33]. Heron colonies in the western coastal states have been useful for monitoring contaminant concentrations in lakes, rivers, and estuaries. Mercury levels in heron tissue have been measured at a number of sites in the United States and elsewhere, providing a broad basis for comparison [32,34–36]. Numerous other species of birds breed or winter at Clear Lake or use the lake as a resting and foraging stop during migration. We also collected red-winged blackbird (*Agelaius phoeniceus*), Brewer's blackbird (*Euphagus cyanocephalus*), and cliff swallow (*Hirundo pyrrhonota*) nestlings, to learn whether these insectivorous birds were exposed to Hg and MeHg at lower concentrations and to see if an effect of distance from the mine was evident at this lower trophic level. Effects of Hg on raccoons, otters, and mink have been reported in numerous studies [37–48], and are summarized in Wolfe et al. [49]. These studies provide a basis for comparison and interpretation of the tissue Hg residues in animals we sampled at Clear Lake. Of the other species used in the GLWQI model, osprey were subject of a concurrent study [50], bald eagles are present at Clear Lake only seasonally and are restricted, and belted kingfishers are customarily collected by shotgun, a difficulty in a populated area. Although the GLWQI model was developed for a system in which the Hg source is atmospheric deposition, the methodology used is independent of source criteria; therefore, distance from an Hg point source was not one of the variables of interest. However, because the relationship between distance from the mine and Hg concentration in the biota was a focus of the U.S. EPA Region Superfund/UC–Davis study, we included it in our analysis whenever practical. Because the great blue heron colonies occurred at various distances from SBMM, herons were a particularly useful species to monitor in support of this secondary objective as well. Raccoons were usually common all around the lake in 1993, although an epizootic of canine distemper in the summer and fall of 1993 reduced raccoon numbers available for sampling in 1994. Mink and otter are found in wetlands and along creeks and sloughs. Observations by residents and local biologists suggest that otter and mink populations have increased during the last 10 years, but no formal population studies have been conducted.

METHODS

Sample collection

All species sampled were collected (or captured, sampled, and released) under U.S. Fish and Wildlife Service permit PRT-779203, California Department of Fish and Game (CDF&G) permit 4017, and Memoranda of Understanding with the CDF&G for carnivores.

We collected heron, cormorant, blackbird, and cliff swallow nestlings from nests. Recently dead and dying heron and cormorant nestlings were salvaged after falling from nests. Raccoons and mink were taken in box traps or padded double-swivel leg-hold traps and either sacrificed, or anesthetized, sampled, recovered in the field, and released.

Blood, brain, muscle, kidney, liver, claws, and fur or feathers were collected from all animals sacrificed. Blood, claws, and fur or feathers were collected from live-sampled animals. Brains of a subset of animals were separated into right and left hemispheres, one half was frozen for Hg analysis, the other

was preserved in formalin solution and sent to the Veterinary Pathology Laboratory at Oregon State University (Corvallis, OR, USA) for histopathologic examination.

We sampled food items from the ground below nests (herons and cormorants), from water and air near nests (insectivorous passerines), or from the gastrointestinal tracts of collected animals. We determined heron food energy requirements according to the method of Butler [51], then used these values and food item Hg concentration to determine actual Hg exposure in heron young. We then compared the model's predicted toxicity threshold water concentration to measured Hg concentration in Clear Lake water.

Residue analysis

Samples for residue analysis were frozen and shipped to Brooks-Rand (Seattle, WA, USA) for total Hg or MeHg analysis by cold-vapor atomic absorbance spectroscopy according to the methods of Bloom and Liang [26,27]. Tissue samples from passerine birds were prepared for Hg analysis by pooling tissues from three to six individuals. Feather samples consisted of flight feathers from the left wing and contour feathers from the left side of the breast, pooled and digested to homogeneity. Liver, brain, and feathers or fur were analyzed for total Hg and/or MeHg. A subset of kidney, blood, and muscle samples also was analyzed for MeHg. Because Clear Lake historically has been contaminated with OCs, we submitted a small subset of Clear Lake bird and mammal tissues to Columbia Laboratories (Corbett, OR, USA) for OC analysis, to compare present-day OC tissue concentrations with those reported historically. We used fillets for the fish analyses, and liver tissue from other animals, and fat-normalized the results, thus permitting comparison between Clear Lake animals and results from previous work [52].

Tissue calibration

We looked for correlations between tissues that can be obtained nonlethally (feathers or fur, and blood and/or claws as available) with Hg concentrations in tissues of toxicologic interest (brain and liver) via regression analysis and t tests.

Growth rates and reproductive effects

We observed breeding activities at the Clear Lake heronries to collect data on incubating nests, young per nest, and young per successful nest [30]. During the incubation period, we counted attended nests using a spotting scope from observation points outside the colonies. Later, when the colonies could be entered without undue disruption, we counted young in the nest from the ground beneath trees [53]. The professional climber who collected the nestlings for tissue analysis made final counts of young in nests on collection day. We took morphologic measurements (weight and body length; and for herons and cormorants, culmen, wing, and tarsus length) of collected animals. We calculated heron nestling growth rates by determining age from culmen length and plotting body weights against the resulting age curve to compare growth rates of Clear Lake heron chicks to growth rates of chicks not exposed to contaminants [29,54].

Biomarkers and bioaccumulation

Mercury and MeHg may inhibit cholinesterase (ChE) activity, or inhance the ChE-inhibiting action of organophosphorus (OP) insecticides [55–58]. Because the Quercus Point rookery was located next to an orchard where guthion was applied, and exposure from drift was a possibility [59], we measured blood and brain ChE activity in nestlings from that colony, and from Slater Island, a colony with no known OP exposure, using the oxime reactivation technique, which allows the animal to serve as its own control [60,61]. Because various blood parameters may be affected by exposure to MeHg [62–64], we analyzed white blood cell ratios on a subset of the animals sampled in 1993. Suchanek et al. [14,21] proposed a bioaccumulation scheme for Hg in Clear Lake based on the compartments they sampled: water, sediment, invertebrates, and fish. We modified the scheme to include the species we sampled, thereby adding two additional trophic levels, and calculated the bioaccumulation factors for each, using our data and the measurements of Suchanek and coworkers.

Statistics

The statistical functions in Excel 4.0 (Microsoft Corporation, Redmond, WA, USA) were used for summary statistics, tests for normal distribution, t tests, and analysis of variance (ANOVA). Regressions were done in Graph III (Computer Associates, San Jose, CA, USA) or Excel.

RESULTS

Hg residues

Great blue herons and double-crested cormorants. With few exceptions, all the food items and parts recovered from nests, the ground below nests, and the gastrointestinal tracts of heron and cormorant young were the Sacramento hitch (*Lavinia exilicauda*). The mean Hg concentration in whole Sacramento hitch was 0.563 ppm (0.31–0.87 ppm). Because 90 to 99% of the Hg in fish tissue is in the methyl form [65], this is equivalent to a concentration of approximately 0.3 to 0.85 ppm MeHg in the diet of nestlings fed Sacramento hitch. Mean brain tissue Hg concentration of heron young was 0.35 ppm (0.3–0.4 ppm). Mean liver Hg concentration of heron young was 1.46 ppm (1.32–1.71 ppm). For cormorant young the corresponding values were brain: 0.63 ppm (0.54–0.72 ppm) and liver 2.42 ppm (1.91–2.94 ppm). These values are well below tissue Hg concentrations associated with Hg toxicity in young birds, 1 to 10 ppm in brain and 5 to 20 ppm in liver [49,66]. No correlation was found between tissue Hg concentration and distance from the mine. Tissue Hg concentrations for great blue heron and double-crested cormorant nestlings and their food items are given in Tables 1 and 2. The relationship between heron blood Hg and brain, kidney, and liver Hg was significant ($p = 0.025$). In herons sampled in 1993, no correlation was found between feather Hg and either liver or brain Hg. In 1994, after we improved the feather sampling and analysis method, we found a correlation between feather Hg and liver Hg ($p = 0.01$) but not between feather Hg and brain Hg. In cormorants, a strong correlation ($p = 0.005$) was found between feather Hg and both liver and brain Hg (Fig. 2). No correlation was found between age or body weight and Hg residue in any heron brain or liver.

Insectivorous passerine young. As anticipated, Hg residues (0.018–0.03 ppm in brain, 0.094–0.364 ppm in feathers, 0.24–0.92 in liver) were below known toxicologic benchmarks for all ages of all three species. Thresholds for Hg toxicity in adult birds are 5 to 20 ppm in brain and 9 to 30 ppm in liver [49,66]. Samples of insect food collected from passerine foraging areas contained 0.01 to 0.031 ppm Hg in sweep samples and 0.254 to 0.420 ppm in dip samples. Based on our small sample, the

Table 1. Total mercury (ppm wet wt., mean with standard deviation in parentheses) in tissues of great blue heron young collected at Clear Lake, California, USA

Location	Distance from SBMM[a] (miles)	Year	*n*	Brain	*n*	Liver	*n*	Feathers	*n*	Blood	*n*	Kidney	*n*	Food
Slater Island	5 (8 km)	1993	8	0.36 (0.15)	8	1.42 (0.67)	7	2.23 (0.77)	4	1.3 (0.23)	5	1.15 (0.10)	3	0.87 (0.80)
		1994	6	0.30 (0.14)	11	1.39 (0.58)	11	1.97 (1.18)					7	0.46 (0.12)
Quercus Point	9 (14.5 km)	1993	7	0.35 (0.11)	7	1.71 (0.63)	6	2.43 (0.99)	4	1.08 (0.32)	3	1.00 (0.33)	3	0.31 (0.16)
		1994	6	0.36 (0.15)		1.46 (0.57)	11	2.01 (1.18)					4	0.44 (0.17)
Rodman Slough	14 (22.5 km)	1993	10	0.4 (0.09)	10	1.32 (0.59)	10	3.16 (0.82)	5	1.16 (0.33)	7	1.12 (0.26)	4	0.46 (0.04)

[a] Sulphur Bank Mercury Mine.

proportion of MeHg in both liver and brain total Hg was approximately 84%. Tissue Hg concentrations for red-winged blackbird, Brewer's blackbird, and cliff swallow young and their food items are shown in Table 3. A relationship was apparent between brain Hg in passerine young and distance from the mine ($r^2 = 0.54$), but sample sizes were too small for confidence. An apparent correlation exists between feather Hg and brain and liver Hg, when passerine species were considered together, but data were insufficient to determine a relationship for individual species.

Raccoons. Raccoon results were grouped into three sets based on distance from SBMM (Table 4). The most notable characteristic of Hg tissue concentration in Clear Lake raccoons is variability, which probably reflects the raccoon's omnivorous, opportunistic feeding habits. Brain Hg concentrations (ppm) of all raccoons collected were below NOAELs and LOAELs reported for wild mammals [46,67,68]. The highest brain and liver Hg concentrations, 1.15 ppm and 8.46 ppm, respectively, were found in a raccoon collected 14.5 km (9 miles) from SBMM; surprisingly this animal had only 0.46 ppm Hg in its fur. When this raccoon was included in the tissue calibration analysis, no correlation was found between fur and brain Hg concentrations, but removing this animal from the data set gave a significant fur–brain Hg correlation ($p = 0.025$). We found no correlation between blood or fur Hg concentrations and liver Hg. A strong correlation ($p = 0.005$) was found between claw and liver Hg concentrations, but no correlation was found between claw and brain Hg. Raccoon body burden of Hg did not correlate with body weight or with distance from the mine.

Mink. A total of eight mink were sampled in the 1993 and 1994 field seasons, from approximately 250 trap sets. The highest brain Hg concentration, 7.1 ppm in a mink collected at the north end of the lake 22.5 km (14 miles) from the mine, is notable (Table 5). This is the only tissue Hg concentration collected at Clear Lake that was close to a toxicity threshold reported for wildlife species. Brain Hg concentrations associated with toxic effects in adult mink range from 8 to 30 ppm [46,49,66].

OC analysis

Thirty-five samples from Clear Lake animals had liver dichlorodiphenyldichloroethylene (DDE) residues less than 0.4 ppm. One heron nestling had 2.54 ppm DDE in its liver. Six herons and four fish also had tetrachlorodiphenylethane (TDE) residues above the detection limit of 0.3 ppm. Organochlorine tissue residues from this study are compared with OC tissue residues from animals measured in the 1950s and 1960s in Table 6.

Growth rates and reproductive effects

In 1993, the median age of the nestlings was 29.6 d (20–71 d). In 1994, the median age of young collected was 31.4 d (18–57 d). This spread of ages permitted construction of growth curves. No significant difference was found between growth rates of great blue heron nestlings from Clear Lake heronries compared with growth rates for nestlings from colonies in Washington [69] and Nova Scotia [29] (Fig. 3). Growth rate of Clear Lake heron young also did not differ from herons raised in captivity with unlimited food [54]. These data indicate that heron young sampled at Clear Lake in 1993 or 1994 did not have impaired growth, when compared to the growth rate of heron young for sites uncontaminated by Hg.

In 1993 we counted 121 active heron nests at Rodman Slough. Counts of young per successful nest made during the nesting period and again when chicks were collected yielded an estimate of more than two fledglings per nest. The Rodman Slough colony had 93 active nests in March of 1994, but the colony was abandoned between April 2 and April 15 (the herons have since returned). The Slater Island colony contained 65 active nests in 1993 but only 45 in 1994, although the colony has expanded to the west end of the island in recent years [70]. In contrast to 1993, when breeding was very synchronous, in 1994 eggs and young were at various stages of development in the colony at each observation period between April 2 and May 13. We estimated two fledglings per successful nest in 1993. In 1994, 1.55 young were found per active nest based on a count of nests that could be observed without

Table 2. Total mercury (ppm wet wt., mean with standard deviation in parentheses) in tissues of double-crested cormorant young collected at Quercus Point, Clear Lake, California, USA

Year	*n*	Brain	*n*	Liver	*n*	Feathers	*n*	Food
1993	5	0.72 (0.15)	5	2.94 (1.07)	5	2.95 (0.31)	3	0.31 (0.16)
1994	10	0.54 (0.09)	10	1.91 (0.39)	10	4.05 (1.32)	4	0.38 (0.29)

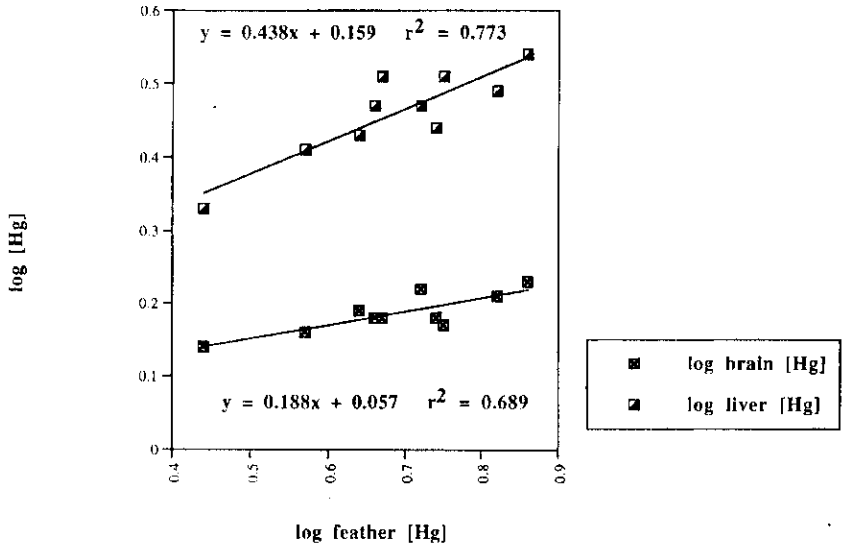

Fig. 2. Double-crested cormorant feather mercury (Hg) concentration versus brain and liver Hg concentration.

disturbing the colony and a final count made at the time of collection. Double-crested cormorants were the most numerous species nesting at Quercus Point, with an estimated 210 nests. More than 130 great blue heron nests, 3 black-crowned night-heron nests, and an osprey nest were found at Quercus Point. Heron and cormorant nests were distributed among mixed oak and cottonwoods; both species often were found in the same tree. In 1993, more than 100 active heron nests

Table 3. Mercury (Hg) and methylmercury (MeHg) residues (ppm wet wt.) in Clear Lake, California, USA, insectivorous passerine young and their food

Species	Distance from SBMM[a] (miles)	Sample/tissue[b]	Total Hg only (ppm wet wt.)	MeHg (ppm wet wt.)	Total (MeHg + HgII)	MeHg/total Hg
Red-winged blackbird						
8–12 d	0.5 (0.8 km)	Pooled brains	0.029			
		Pooled livers	0.092			
		Pooled feathers	0.094			
1–7 d		Pooled brains		0.027	0.030	0.90
		Pooled livers		0.068	0.082	0.83
		Pooled feathers	0.364			
		Pooled food (d)	0.420			
		Pooled food (s)	0.031			
	5 (8 km)	Pooled livers	0.037			
		Pooled food (d)	0.254			
		Pooled food (s)	0.012			
Brewer's blackbird	5 (8 km)	Pooled brains	0.023			
		Pooled livers	0.044			
		Pooled feathers	0.136			
		Pooled brains		0.027	0.030	0.89
		Pooled livers		0.019	0.024	0.79
		Pooled food (s)	0.010			
Cliff swallow (Clear Lake State Park)	8 (12.9 km)	Pooled brains	0.021			
		Pooled livers	0.049			
		Pooled feathers	0.322			
		Pooled brains		0.006	0.007	0.81
		Pooled livers		0.035	0.042	0.83
	8 (12.9 km)	Pooled food (s)	0.017			
Rodman slough	14 (22.5 km)	Pooled brains	0.018			
		Pooled livers	0.074			
		Pooled feathers	0.159			

[a] Sulphur Bank Mercury Mine.

[b] Each tissue value is a single pooled sample consisting of tissues from three to six individuals. Food samples were collected with dip (d) or sweep (s) nets from areas where birds were observed foraging.

Table 4. Mercury (Hg) tissue concentrations (mean with standard deviation in parentheses) in raccoons collected at Clear Lake, California, USA

Distance from SBMM[a] (miles)	Total Hg (ppm wet wt.)				
	Brain	Liver	Fur	Blood	Claws
0.1–0.5 (0.2–0.8 km)	0.67 (0.35)	3.29 (5.1)	21.97 (13.2)	0.40 (0.20)	10.22 (16.7)
6 (9.7 km)	0.15 (0.08)	1.02 (0.58)	6.93 (8.4)	0.07 (0.05)	3.74 (2.1)
7–10 (11.3–16.1 km)	0.63 (0.56)	7.02 (5.9)	4.05 (5.8)	0.24 (0.20)	13.0 (13.1)

[a] Sulphur Bank Mercury Mine.

and an estimated two to three fledglings per nest were found. In 1994, we counted 50 active nests at Quercus Point. The number of fledging-age young could be confirmed in 21 of these nests, which yielded a mean of 1.47 fledglings per active nest. As with Slater Island, in 1994 breeding was asynchronous, with eggs and young at various stages of development throughout the observation period. Based on our observations, the reproductive success rates of great blue herons at Clear Lake appear to be comparable to success rates of great blue heron colonies from nearby sites not contaminated with Hg [71,72].

Biomarkers and bioaccumulation

Brain of raccoons and mink were examined at Oregon State University's Veterinary Pathology Laboratory for signs of Hg-induced lesions. No histopathologic changes were observed.

Blood cell smears of a subset of bird and mammal blood samples taken in 1993 did not indicate changes in erythrocyte morphology [73] or elevations indicative of chronic infection [74] (Table 7). We did not have blood cell counts for the same species from reference sites, so no conclusions can be drawn about Hg-related alterations in white cell proportions [64,75], but we found no correlation between Hg tissue concentration and heterophil to lymphocyte ratio. Western grebes sampled by Elbert [76] at Clear Lake and at Eagle Lake, a site uncontaminated by Hg, did show a difference in white cell populations between the two sites, but the difference was not statistically significant. The heterophil to lymphocyte ratio of herons (approximately seven to three), the reverse of the ratio in the two passerine species (approximately two to seven for cliff swallows and two to six for red-winged blackbirds) is apparently a characteristic of the Ciconiiformes [77].

Eggshell thickness

Eggshell thickness measurements from Clear Lake heronries and from heronries uncontaminated by Hg, show that eggshell thickness was at pre-DDT/DDD levels, suggesting that Hg or the combination of Hg and OCs has not caused shell thinning. Observations under the colonies found no evidence of hatching failure or predation, and no eggshells collected were fragile or cracked, findings typical of OC effects.

ChE activity

Brain and plasma ChE activity measurements indicate that ChE activities were not depressed in either the Quercus colony, in which nestling herons possibly were exposed to both Hg and guthion, or at Slater Island where there was no indication of OP exposure, indicating that neither Hg or the combination of Hg and possible guthion exposure at the Quercus colony was sufficient to depress ChE activities. Pre- and postreactivation activities were within 3%. Brain ChE activities of Quercus Point and Slater Island herons were 12.9 and 11.4 μmoles acetylthiocholine (AcThCh) hydrolyzed per gram brain weight, respectively. Plasma acetylcholinesterase (AChE) activities of Quercus Point and Slater Island herons were 0.25 and 0.27 μmoles AcThCh hydrolyzed per ml plasma, respectively.

Figure 4 shows exposure pathways and associated Hg concentrations for a site on Oaks Arm close to SBMM. Trophic relationships are based on analysis of stomach contents or on the known biology of the species. Bird and mammal values are liver Hg concentration, ppb wet weight. The water : hitch (trophic level 2 [TL-2] BAF is 11,100 and the water : heron (TL-3) BAF is 31,200, based on Oaks Arm water Hg concentration of 5.08×10^{-2} ppb, hitch mean Hg of 563 ppb, and heron liver mean Hg concentration of 1,590 ppb. The water : mink BAF

Table 5. Mercury (Hg) concentrations in the tissues of mink collected at Clear Lake, California, USA

Body weight (kg)	Distance from SBMM[a] (miles)	Tissue total Hg (ppm wet wt.)						
		Brain	Liver	Fur	Blood	Muscle	Kidney	Claws
1.45	0	0.12	10.1	3.28	0.63			10.2
1.36	7 (11.3 km)			7.49	0.21			11.9
0.45	7 (11.3 km)			2.99	0.08			
1.74	14 (22.5 km)	7.10	9.25	21.5	0.18			34.1
1.21	14 (22.5 km)	0.24	0.93	7.99	0.16	0.58	0.67	4.70
0.67	14 (22.5 km)	0.40	2.26	6.38		1.10	1.30	7.22
1.15	14 (22.5 km)		0.44	2.13		0.28	0.405	2.28
1.06	14 (22.5 km)	0.29	1.30	7.63	0.15	1.13	1.03	5.26

[a] Sulphur Bank Mercury Mine.

Table 6. Organochlorine residues (mean with standard deviation in parentheses) in the tissues of Clear Lake, California, USA, wildlife sampled in 1993 compared to 1958–1963

Species	1958–1963 (ppm in fat)	*n*	Liver 1993 (ppm)	Liver Fat (%)	Liver Fat equivalent (ppm)
Mink		1	0.07	1.63	23.3
Raccoons		7	0.048 (0.028)	2.09	43.5
Great blue herons		19	0.079 (0.116)	2.36	29.8
Western grebe	321–2,633				
Goldeneye	132				
Double-crested cormorants		3	0.204 (0.266)	2.03	9.95
Gull species	118–2,134				
Cliff swallows		1	0.15[a]	6.58	43.8
Brewer's blackbird		1	0.18[a]	5.57	30.9
			1993 (ppm in edible tissue)	% Fat	Fat equivalent for edible tissue[b]
Carp	40				
Sacramento hitch		5	0.049 (0.058)	6.10	0.90

[a] Pooled sample.
[b] [3].

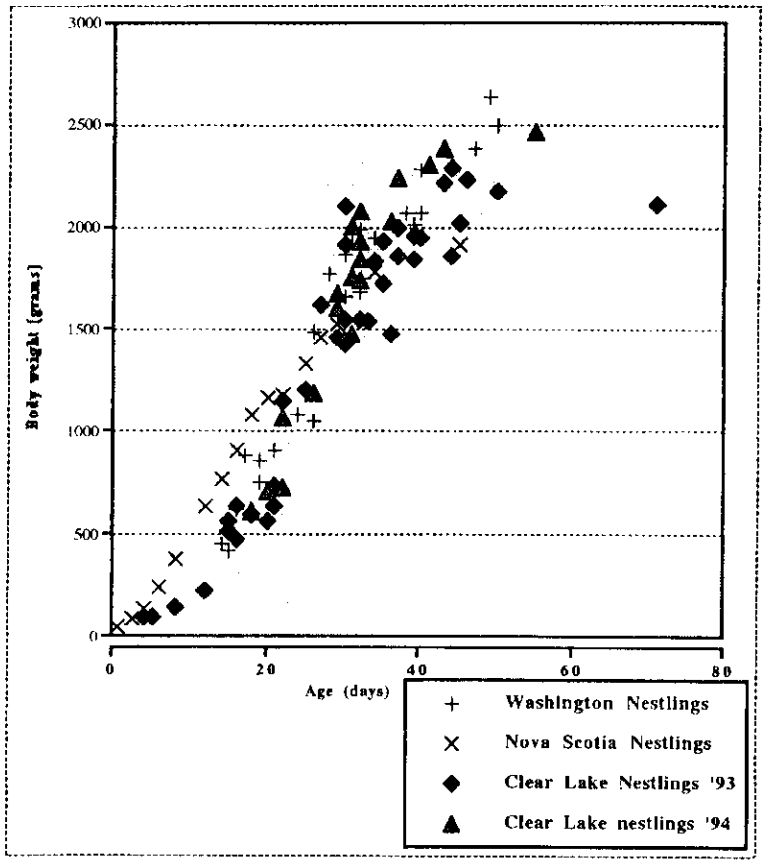

Fig. 3. Growth curves of great blue heron nestlings at Clear Lake, California, USA, compared to nestlings not exposed to mercury.

Table 7. White blood cell counts (mean with standard deviation in parentheses) of birds collected in Clear Lake, California, USA

Sample	*n*	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Total mercury (liver)
Great blue herons							
Quercus Point	9	62.1 (12.6)	34.9 (12.3)	1.4 (1.0)	1.9 (1.0)		1.46 (0.57)
Slater Island	9	71.8 (9.4)	24.4 (8.1)	2.5 (2.5)	2.4 (0.7)		1.39 (0.58)
Rodman Slough	5	65.3 (3.3)	30.0 (2.9)	3.0 (0.0)	4.7 (3.1)		1.63 (0.84)
Red-winged blackbirds	6	21.8 (15.2)	59.3 (16.6)	1.8 (0.8)	4.0 (1.1)	39.5 (4.9)	0.070 (pooled sample)
Cliff swallows							
Rodman	4	23.8 (4.6)	75.0 (5.6)	1.3 (0.6)	1		0.074 (pooled sample)
Cliff swallows							
Clear Lake State Park	13	23.6 (8.8)	73.2 (8.9)	2.5 (1.1)	2.3 (1.7)		0.049 (pooled sample)
Western grebe							
Clear Lake	8	54.4 (10.2)	33.8 (9.1)	1.6 (1.4)	10.1 (5.3)		
Eagle Lake[a]	5	40.4 (14.4)	41.8 (12.2)	3.8 (2.2)	13.8 (4.1)		

[a] Reference site uncontaminated with mercury.

(TL-4) is 190,600. Raccoons, with their opportunistic and variable diet, were assigned to a mixed TL-3/4; the water : raccoon BAF is 73,500.

DISCUSSION

Budgetary and logistic constraints imposed several problems on the study design. (1) The study had no controls, and no matched reference population. All parameters could be compared only to conspecifics in uncontaminated sites, and/or to values reported in the literature. (2) Sample sizes were small. Only great blue herons were represented by a sample sufficient to achieve statistical power. (3) Formal randomization was not achievable. Although we made efforts to distribute sample sites throughout the bird colonies, and to trap mammals from multiple locations around the lake, in practice sampling patterns and sample sizes were dictated by accessibility, regulatory constraints, and budget. Therefore, to increase confidence and help to compensate for these shortcomings, we maximized the number of parameters assessed, measuring everything that might reflect a Hg exposure, within the severe constraints of our budget. Our reasoning was that, because the sample sizes collected were necessarily smaller than optimum, we should obtain the maximum informational benefit from each sample.

Care must be taken in comparing eggshell thickness measurements between herons from different regions because of recognized differences in shell thickness among subspecies. The sample of eggshell thickness measurements we took served mainly to reinforce our observation that shell breakage was not a cause of nest failure in Clear Lake heronries. This result is consistent with the low OC concentrations found, and our examination of shells collected in the colonies. Unfortunately, we were unable to obtain OC tissue residue analyses for the same species and tissues that were measured in the 1950s and 1960s. However, even the across-species comparisons in Table 6 show a gratifying decline in OC body burden during the intervening 25 to 30 years.

Cahill and coworkers [78] also measured Hg residues in feathers of Clear Lake birds. They used the distal 7 to 9 cm

Table 8. Great Lakes Water Quality Initiative (GLWQI) parameters compared to Clear Lake, California, USA measurements[a]

		GLWQI	Clear Lake	Clear Lake–Oaks Arm (CL-OA) (pg/L)	Clear Lake–Lower Arm (CL-LA) (pg/L)	CL-OA/GLWQI	CL-LA/GLWQI
WV toxicity threshold, water Hg concentration		1,300 pg/L		51,200	6,550	39	5
Avian Hg NOAEL (with *UF* of 20)		0.0032 mg/kg/d					
Avian Hg NOAEL (without *UF*)		0.064 mg/kg/d					
Food Hg concentration (Sacramento hitch)			0.56 mg/kg (ppm) (0.3–0.85)				
Dietary intake (great blue heron nestlings)			0.138 mg/kg/d (0.073–0.21)				
TL-2 BAF	Hitch GBHE[b] nestlings			11,000	85,954		
TL-3 BAF		27,900 L/kg		28,500	223,000		
TL-3/4 BAF	Raccoon			72,800	570,000		
TL-4 BAF	Mink	140,000 L/kg		189,000	1,478,000		

[a] WV = wildlife value, Hg = mercury, NOAEL = no observed adverse effect level, *UF* = uncertainty factor, TL = trophic level, BAF = bioaccumulation factor.
[b] GBHE = Great blue heron.

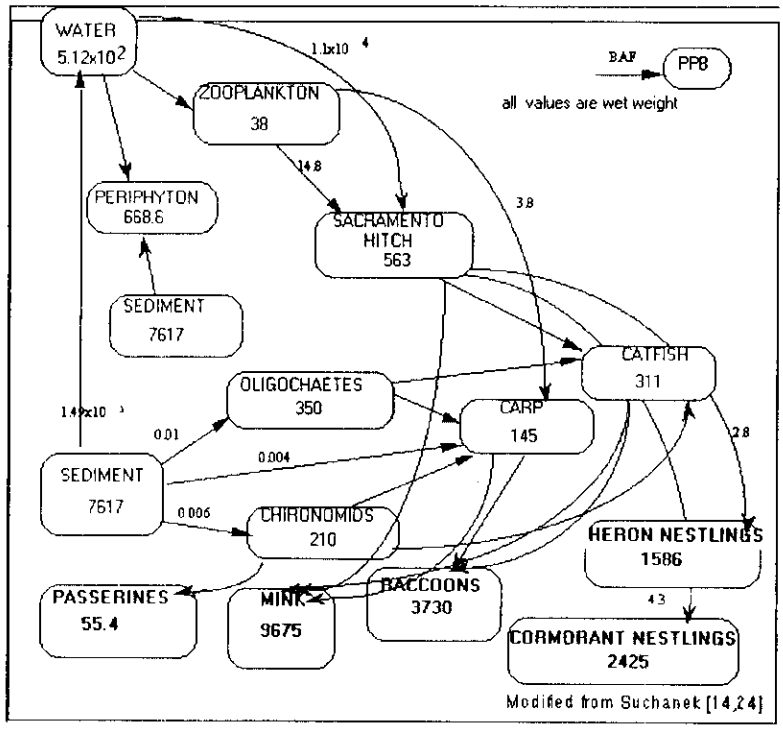

Fig. 4. Bioaccumulation of total mercury (Hg) in Clear Lake, California, USA, biota.

of a primary flight feather for their analysis, rather than the pooled flight and contour samples we used, and reported 6.48 ppm Hg for juvenile cormorant feathers, compared to our 2.95 ppm in 1993 and 4.05 in 1994. The heron feather samples of Cahill and coworkers contained 7.4 ppm Hg compared to 1.97 to 3.16 ppm for feathers from herons in our study, in both years at all three sites. However, Cahill and coworkers collected feather samples from adult herons, whereas we sampled only young herons.

Because of the proximity of the Quercus Point heron colony to an orchard treated with an OP during the nesting season, we were afforded an opportunity to assess another possible additive interaction of two contaminants that individually occurred at concentrations too low to cause health effects, and also to take into account a potentially confounding variable. Quail receiving the dietary concentration of MeHg (5 ppm) sufficient to depress ChE activity (without coadministered parathion) had liver total Hg residues of 35.8 ppm, wet weight [55,79]. Clear Lake great blue heron nestlings, fed fish containing 0.3 to 0.9 ppm Hg, and accumulating 1.3 to 1.7 ppm Hg in the liver did not exhibit depression of ChE activity in brain or plasma. However, this dietary concentration and liver residue were, in the study of Dieter and Ludke [55,79], sufficient to potentiate the ChE-inhibiting action of a carbamate or OP insecticide to which the quail were simultaneously exposed.

The Hg exposure of young insectivorous passerines feeding on emergent aquatic insects was low, and resulting tissue residues were also low. However, our interest in insectivorous passerines arose in part because of what they might reveal about the exposure of bats, small mammals that share the aquatic insectivore niche. Bats were not considered in the GLWQI model, which regards upper trophic level piscivores as the species most at risk. We believe that potential damage to bats should always be considered when assessing risk or deriving standards for waterborne contaminants, especially those that bioaccumulate. Bats are small, with body weights from 3 to 30 g. They have high food consumption rates to meet the energy demands of flight. They are long-lived, with life spans of 10 years or more, and are therefore potentially at greater risk from contaminants that bioaccumulate and bioconcentrate. Bats have low reproductive rates, typically one or two young per year; thus bat populations may be more severely harmed by reproductive toxicants. A bat of 10 g body weight, and 1 g/d food intake rate, if feeding on insects with total Hg concentrations found in Clear Lake invertebrates, would be ingesting 5 to 20 times the mammalian Hg NOAEL used in the GLWQI model [5,80–82].

The lack of a correlation between raccoon tissue Hg concentration and feather or fur Hg concentration is quite different from the findings of Roelke et al. [45]. In their survey of prey of the Florida panther, they found a close correlation between tissue Hg and fur Hg concentrations in raccoons. We suggest that Everglades raccoons occupying the range of the Florida panther are feeding throughout the year on a more natural diet of fish, crustaceans, and bird eggs in which Hg is more homogeneously distributed, whereas Clear Lake raccoons whose foraging ranges include residential, recreational, and agricultural land consume an opportunistic diet in which Hg content varies greatly, not only seasonally, but from day to day. The temporal relationship between Hg tissue distribution and time of exposure has been reported by a number of investigators [83]. Raccoons were abundant all around the lake 1993, although an epizootic of canine distemper in the summer and fall of 1993 reduced raccoon numbers available for sampling in 1994. The usual abundance of raccoons at Clear Lake and their ease of capture would make them good candidates for Hg monitoring in Clear Lake mammals, but the variability of

Hg exposure and ingestion reflected by our raccoon data reduces their suitability for that purpose.

Mink and otter are found in wetlands and along creeks and sloughs. Observations by residents and local biologists suggest that otter and mink populations have increased during the last 10 years, but no formal population studies have been conducted. Therefore, mink would be a better choice for monitoring Hg in wild mammals at Clear Lake, but unfortunately are much more difficult to sample. Many more mink were caught than were sampled, as they often can pull out of the padded leghold traps. The highest brain Hg concentration of an animal at Clear Lake, 7.1 ppm in an adult mink, falls between the brain Hg concentrations associated with the dietary NOAEL and LOAEL reported for mink by Wobeser et al. [46,67]. After 120 d on a diet containing 0.44 mg/kg/d of MeHg, mink exhibited no clinical or pathologic signs of Hg intoxication and had brain Hg concentrations of 3.4 ppm. Mink receiving 1.1 mg/kg/d for 93 d displayed no clinical signs but were found on necropsy to have nerve tissue lesions. Brain Hg residues in these mink were 8.2 ppm [46,67].

Tissue calibration results for feathers and blood were promising. Given the ease with which feathers can be obtained from birds, their use as a monitoring tool for Hg and other heavy metals is very attractive. We hope to conduct laboratory investigations that will further refine methods for using feathers as a heavy metal monitoring tool in birds. Our correlations between blood and body tissues were better, but although blood can be obtained nonlethally, it is certainly more invasive a sample to obtain than feathers.

The avian species sampled were not the same as those used in the GLWQI model, belted kingfisher, bald eagle, and herring gull. The strength of the model is therefore further tested by applying it to species that share the Hg-exposure niche (upper level piscivores) but otherwise have different biology than the model species. Heron and cormorant young were fed almost exclusively Sacramento hitch. Because these fish feed on *Daphnia* (14–98% depending on size) and other zooplankton [84], they are assigned to TL-2, making Clear Lake heron and cormorant nestlings TL-3 feeders. The Hg concentration in Sacramento hitch samples we collected, approximately 0.3 to 0.85 mg/kg, yields a dietary intake for heron nestlings of 0.073 to 0.21 mg/kg/d, using a food consumption rate of 0.245 kg/d for a chick with 1.0 kg body weight. The avian LOAEL (TD) derived from the three-generation mallard feeding study of Heinz [85] was 0.064 mg/kg/d, which falls within the range of Hg concentrations in food fed to nestling herons and cormorants at Clear Lake. This value, an LOAEL to NOAEL uncertainty factor (UF_L) of 2, and an interspecies extrapolation factor (UF_A) of 10, yielded the NOAEL used in the GLWQI model, 0.0032 mg/kg/d. The UF_A was incorporated into the NOAEL because of the limited availability of dose–response data derived from chronic controlled feeding studies of MeHg to birds. Given the proximity of the dietary exposure of nestlings herons to the experimental LOAEL, use of the two uncertainty factors would seem justified.

The proposed wildlife criterion for Hg derived from the original, draft version of the GLWQI model was 180 pg/L (1.8×10^{-4} ppb) Hg at the time this study was conducted in 1993. Total Hg in water in the Lower Arm of Clear lake, the cleanest part of Clear Lake, was 6.5×10^{-3} ppb (6,550 pg/L), 36 times greater the initial, proposed GLWQI WV. Total Hg in Oaks Arm, nearest the mine, was 5.08×10^{-2} ppb (50,800 pg/L), 282 times greater than the GLWQI WV. The 1993 draft GLWQI model therefore predicted greater exposure in Clear Lake wildlife than our actual measurements indicated. After public comment, scientific review, and incorporation of additional data, the WV for Hg adopted for final rulemaking was 1,300 pg/L [5]. The Oaks Arm and Lower Arm water Hg concentrations are therefore 39 times greater and 5 times greater, respectively, than the revised, final WV.

Bioaccumulation dominates the WV for contaminants that bioaccumulate. Initially, based on data from the 1-month Clear Lake preliminary study [14], we calculated bioaccumulation factors at Clear Lake that were very different from laboratory-derived values used to calculate the draft GLWQI Hg WV [86]. The upper trophic level piscivores in the GLWQI model were TL-3 or TL-4 feeders. The total Hg water : fish BAFs use for the draft WV for these levels were WV 60,000 and 130,000, respectively. We assigned heron and cormorant young at Clear Lake, feeding on Sacramento hitch, to TL 2/3, based on hitch stomach contents available at the time. The measured water : fish BAF was 71,400, using the preliminary Oaks Arm total Hg concentration of 5.6×10^{-3} and a mean Sacramento hitch Hg concentration of 0.4 ppm. By incorporating data from the expanded study, we derived a water : fish (TL-2) BAF of 11,100 and a water : heron (TL-3) BAF of 31,200, based on Oaks Arm water Hg concentration of 5.08×10^{-2} ppb, hitch mean Hg of 563 ppb, and heron liver mean Hg concentration of 1,590 ppb. Similarly, we obtained a water : mink (TL-4) BAF of 190,600, and a mixed TL-3/4 BAF for water : raccoon of 73,500.

Bioaccumulation factors used to derive the final GLWQI Hg WV were revised to 27, 900 for TL-3 and 140,000 for TL-4. The Clear Lake TL-3 BAF of 31,200 is within 12% of the final GLWQI TL-3 BAF. The final GLWQI TL-4 BAF of 140,000 lies between the TL-3/4 BAF of 73,500 and the Clear Lake TL-4 BAF of 190,600, showing good agreement between the revised model and actual measurements in the Clear Lake system (Table 8).

Although mercury levels are higher in Clear Lake heron and cormorant nestlings than in nestlings sampled from uncontaminated sites, the breeding populations of both species at Clear Lake appear to be stable or increasing. We can make only qualitative comparisons in the absence of background reproductive data for the area. We compared productivity with dozens of other great blue heron colonies [71]. Data from these colonies are limited to young per successful nest, which tends to overestimate productivity because it eliminates failed nests. Our estimates of 1.5 young per active nest are similar to 1.9 per active nest in British Columbia [30]. As we were not monitoring the Rodman Slough herony during the incubation period, when it was abandoned, we can only speculate on the cause of abandonment. When working with colonial nesting birds, protocols for avoiding disturbance while monitoring must be strictly followed; some colonies may not be searched until chicks are 4 to 6 weeks old. We noted that Rodman Slough herons built nests low in oaks, and were more readily disturbed than at the other colonies, where the birds were more habituated to human presence. However, the herons returned to Rodman in 1995 and 1996. Cormorants extended their breeding activities to Rodman in 1995 and 1996 [87].

Our results suggested that the model as originally applied was too conservative for some Hg-bearing aquatic systems, but modifications adapted for the final WV of 1,300 pg/L have made the model more predictive. Further, our findings demonstrate the importance of accurate information on the feeding

and breeding ecology of species of concern in deriving reliable wildlife values from the model [88–92].

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PERGAMON

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Historical and present fluxes of mercury to Vermont and New Hampshire lakes inferred from ^{210}Pb dated sediment cores

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Abstract

Lakes across the Northern Hemisphere have experienced enhanced atmospheric deposition of anthropogenically derived Hg for over 100 years. In the present study, we quantified Hg fluxes to the sediments of ten small drainage lakes across Vermont and New Hampshire, USA, for the period ~1800 to present. Dates were established by ^{210}Pb. Total Hg (HgT) fluxes to sediments ranged from 5 to 17 $\mu g\,m^{-2}\,yr^{-1}$ during pre-industrial times, and from 21 to 83 $\mu g\,m^{-2}\,yr^{-1}$ presently. Present-day HgT fluxes are between 2.1 to 6.9 times greater than pre-1850 fluxes. Current-day direct atmospheric Hg deposition to the study region was estimated at 21 $\mu g\,m^{-2}\,yr^{-1}$, which agrees well with measured HgT deposition, when re-evasion of Hg is accounted for. Our data suggest that Hg fluxes to lake sediments have declined in recent decades, owing to reductions in atmospheric Hg deposition to the lake surface. Watershed export of atmospherically deposited Hg remains elevated relative to present-day deposition rates, which contributes to the impression that Hg retention by watershed soils has declined.

Keywords: Mercury; Paleolimnology; Atmospheric deposition; Sediment; Watershed

1. Introduction

Environmental mercury (Hg) contamination of aquatic ecosystems is a pervasive environmental problem, with potentially severe toxicological consequences for humans and piscivorous wildlife (USEPA, 1997; Evers et al., 1998; National Academy of Sciences, 2000). The majority of Hg contaminating aquatic ecosystems is understood to be anthropogenically derived and atmospherically deposited (Fitzgerald et al., 1998). In poorly buffered, undisturbed lakes, Hg is transported through watersheds by high molecular weight dissolved organic matter, and the proportion of this Hg which is neither methylated nor re-evaded as Hg^0 is deposited to the sediments (Lee and Iverfeldt, 1991; Mierle and Ingram, 1991; Driscoll et al., 1994a; Hurley et al., 2000). Several studies have underscored the importance of watershed size in controlling Hg fluxes to sediments (Engstrom et al., 1994; Mielli, 1995; Lorey and Driscoll, 1999). Wetland area (Driscoll et al., 1994a; St. Louis et al., 1994), land use (Hurley et al., 2000), and pH (Rada et al., 1993) have also been shown to influence delivery of Hg to sediments.

Paleolimnological studies have been used to estimate whole-lake surficial sediment Hg burdens (Gilmour et al., 1992; Rada et al., 1993), and, when coupled with fine-resolution ^{210}Pb dating, to estimate fluxes of Hg to lake sediments for both modern and historical time frames (Ouellet and Jones, 1983; Engstrom et al., 1994; Von Gunten et al., 1997; Hermanson, 1998; Lockhart et al., 1998; Lorey and Driscoll, 1999). Numerous multiple lake-sediment studies show anthropogenic Hg contamination to be a recent phenomenon (~1850 to present), coincident with industrialization, and fossil fuel and waste combustion (Landers et al., 1998; Pirrone et al., 1998). Engstrom and Swain (1997) have shown that Hg deposition to lakes down-gradient of Midwestern urban centers is declining in response to recent reductions in

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Hg emissions. While there exists significant uncertainty in the estimation of Hg fluxes to individual lakes (Mielli, 1995; Gottgens et al., 1999), the pattern evident in so many paleolimnological Hg studies is clear: anthropogenically derived Hg has increased by a factor of 2–8 × in the sediments of lakes throughout the Northern Hemisphere (Landers et al., 1998).

In the present study, we analyzed a series of single, short-cores taken from undisturbed lakes in Northern New England, to evaluate four specific hypotheses: (1) that Hg fluxes have increased proportionally to increases observed in other studies; (2) that Hg fluxes have decreased in recent years; (3) that Hg fluxes increase with increasing watershed area to lake area ratio; and, (4) that paleolimnologically inferred atmospheric total Hg deposition estimates compare well with measured total wet + dry Hg deposition.

2. Methods

2.1. Site characteristics

The lakes selected for this study lie within the borders of Vermont and New Hampshire, and are characteristic of undisturbed lakes within the Northeastern Highlands Ecoregion (Omernik, 1987; USEPA, 2000). All are small, 8.1–38.9 hectare drainage lakes occupying undisturbed forested catchments, which are a mix of deciduous or coniferous vegetation overlying soils ranging from stony to silty loams. Bedrock geology is largely schistose or granitic, and most watersheds are poorly buffered. Some shales and slates are in evidence near High Pond in Vermont, and the buffering capacity of this watershed is enhanced accordingly. These watersheds have experienced varying degrees of deforestation during settlement, but have regrown to forest in the past 75–150 years. Limnological attributes of the study lakes are provided in Table 1, and their location across the study region is shown in Fig. 1.

2.2. Field techniques

Sixty cm by six cm diameter lexan coring tubes were prepared for sampling by cleaning in a commercial laboratory dishwasher with Alcanox®, followed by soaking in 10% HNO_3, copious rinsing with ASTM Type-II deionized water, and air drying in a metal-free hood. In the field, two sediment cores were acquired from the lake's deep hole using a Glew-design gravity corer (Glew, 1989). The core reflecting the least disturbed stratigraphy and most distinct sediment-water interface was selected for sectioning immediately in the field, which minimized disturbance. Subsamples were extruded at 1-cm intervals to the core bottom, and split, with one half used for Hg analysis, and the other for

Table 1
Location and limnological attributes of 10 Vermont and New Hampshire lakes used to estimate current and historical mercury deposition

Lake	Lat. DDMMSS	Long. DDMMSS	Lake area (Ha)	Basin area (Ha)	Max. depth (m)	Mean depth (m)	Volume (m^3)	Flushing rate (#/yr)	Alkalinity (meq)
Dudley	430730	715030	12.1	673.4	6.1	3.7	444,183	7.8	121.0
Gilman	433030	711200	13.0	255.4	5.2	2.1	276,380	5.0	146.0
High	434510	730914	8.1	70.0	16.0	7.9	641,598	0.5	1,195.0
Intervale	434730	713130	17.4	466.2	14.9	7.0	1,220,269	1.9	94.0
McConnell	444904	714806	35.2	1465.4	5.5	2.3	809,805	17.3	160.0
Sessions	444220	711150	14.2	207.2	10.3	4.9	690,951	1.5	124.0
Spring	432942	725512	26.7	111.3	24.0	10.7	2,850,174	0.7	737.0
Wallingford	432341	725432	35.2	594.9	7.0	2.1	749,950	20.2	114.0
Wheeler	444230	713829	26.7	1683.1	10.0	4.0	1,055,053	42.3	150.4
Willard	430130	720130	38.9	414.4	17.7	8.0	3,137,500	0.8	31.6

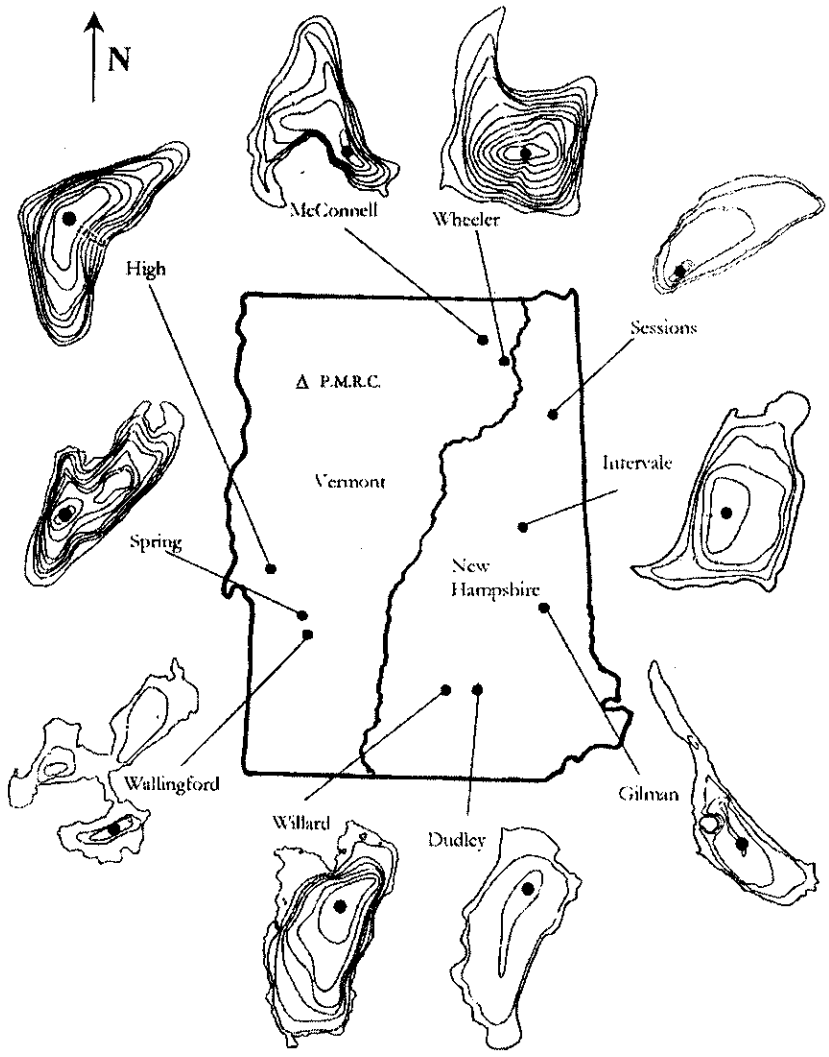

Fig. 1. Geographic location and bathymetry of ten Vermont and New Hampshire lakes used to estimate current and historical mercury deposition. Coring locations are indicated by (●). Map scales differ, and acreages are shown in Table 1. Proctor Maple Research Center (PMRC), an atmospheric monitoring station located in Underhill, VT, is also shown.

^{210}Pb determinations. Mercury-clean sampling procedures (USEPA, 1996) were used throughout the sampling and subsequent sample handling procedures. Sediment aliquots for Hg determinations were stored wet in pre-cleaned, lot-certified 250 ml PETE Nalgene® round vessels, individually bagged in zip-style PETE bags. Sampling was performed during the summer and fall of 1998.

2.3. *Sample processing and analysis procedures*

2.3.1. *^{210}Pb dating*

Sediment cores were analyzed for ^{210}Pb activity to determine age and sediment accumulation rates for the past 150–200 years. Lead-210 was measured at 17–22 depth intervals in each core through its grand-daughter product ^{210}Po, with ^{209}Po added as an internal yield tracer. The polonium isotopes were distilled from 0.3–2.8 g dry sediment at 550°C following pretreatment with concentrated HCl and plated directly onto silver planchets from a 0.5 N HCl solution (Eakins and Morrison, 1978). Activity was measured for $1–8 \times 10^5$ s with ion-implanted or Si-depleted surface barrier detectors and an EG&G Nuclear alpha spectroscopy system. Unsupported ^{210}Pb was calculated by subtracting supported activity from the total activity measured at each level; supported ^{210}Pb was estimated from the asymptotic activity at depth (the mean of the lowermost

samples in a core). Supported ^{210}Pb values for Spring Lake were confirmed by gamma spectrometry on an EG&G Nuclear ultra-low background well-detector. Dates and sedimentation rates were determined according to the constant rate of supply model (OldField and Appleby, 1984) with confidence intervals calculated by first-order error analysis of counting uncertainty (Binford, 1990). All dating analyses were performed at the Science Museum of Minnesota's St. Croix Watershed Research Station.

2.3.2. Mercury in sediment

A small aliquot of homogenized wet sediment was extracted for percent solids determination (APHA, 1999, method 2540B). The remaining sediment was dried at 60°C, a 0.5 g portion of which was then digested in 5 ml aqua-regia for 2 min at 95°C, and brought to 55 ml with ASTM Type-II deionized water. Hg in the sample was converted to Hg^{2+} by oxidation with 15 ml $KMnO_4$, and further brought to 110 ml. This aliquot was mixed with $SnCl_2$ to reduce Hg^{2+} to Hg^0, which was carried by Ar into a Leeman^R automated cold vapor atomic absorption spectrometer (USEPA, 1994, method 245.1 and 245.5). Following initial calibration, standards were run before and after all sample runs, and every tenth sample during the run, as were reagent blanks and matrix spikes. Individual samples were run in duplicate. Standard reference material (SRM, Standard Soil CRM008-050, Resource Technology Corp., Laramie, WY, USA) was analyzed to ensure the completeness of the digestion process. The method detection limit for sediment HgT was 0.05 μg g^{-1}. The analytical accuracy of the mercury data, estimated as relative percent difference between duplicates, was ±1.7%. Analytical precision, estimated as the mean percent recovery for matrix spikes, was 97.4%. The average residual concentration of SRM relative to their certified values was +0.01 μg g^{-1}, representing a mean relative difference of 3.4%. These analyses were performed at the Vermont Department of Environmental Conservation's (VTDEC) LaRosa Environmental Laboratory.

2.3.3. Other parameters

Long-term mean alkalinity values (Table 1) were calculated from available data within the VTDEC Lake Inventory Database and the New Hampshire Department of Environmental Services Lake Trophic Status Database. Original samples were analyzed following standard methods (APHA, 1999). Long-term atmospheric Hg deposition values were measured at the Proctor Maple Research Center (PMRC), in Underhill, VT (Fig. 1), and taken from Scherbatskoy et al. (1999) and Shanley et al. (1999).

2.3.4. Calculations

Fluxes of total Hg to lakes were calculated as the product of the ^{210}Pb-derived sedimentation rates and dry-weight total Hg concentrations, for each core interval, and these are assumed to represent net sedimentation of Hg to the individual lake sediment focal centers. Background fluxes were estimated as the average of pre-1850 fluxes. Linear regressions estimating the relationship between time-averaged Hg fluxes and the ratio of watershed:lake area were calculated using SAS PROC REG. Watershed retention of atmospherically deposited Hg was calculated as the ratio of the regression slope to the regression y–intercept (Engstrom et al., 1994). The variance of this ratio was based on the following algorithm (Mickey, 2001):

$$\mathrm{var}(b/a) = b^2/a^2\{[\mathrm{var}(b)/b^2] - [2\,\mathrm{cov}(a,b)/ab] - [\mathrm{var}(a)/a^2]\},$$

where a is the regression y–intercept, for the independent variable value 0; and, b is the regression slope.

3. Results and discussion

3.1. ^{210}Pb dating and sedimentation rates

For all ten lakes, supported ^{210}Pb concentrations ranged from 0.28 to 2.5 pCi g^{-1}, and the number of deeper core intervals from which supported ^{210}Pb was estimated ranged from one (McConnell Pond) to six (Intervale Pond). Inventories of unsupported ^{210}Pb in the ten cores ranged from 6.77 to 22.02 pCi cm^{-2}, which is equivalent to ^{210}Pb fluxes of 0.22–0.71 pCi cm^{-2} yr^{-1} (Fig. 2 and Table 2). These ^{210}Pb fluxes are similar to regional estimates of atmospheric ^{210}Pb deposition (0.5 pCi cm^{-2} yr^{-1}), which implies that core-specific sedimentation rates are not appreciably amplified by sediment focusing. The underlying assumption for this conclusion is that direct atmospheric deposition dominates the ^{210}Pb budgets of these lakes (i.e., little watershed contribution) (Oldfield and Appleby, 1984). Dates corresponding to the bottom-most unsupported ^{210}Pb strata ranged from 1787 (Wallingford Pond), to 1861 (Spring Lake), with a lake set-wide average value of 1825 (S.D. = 21 yr). Sedimentation rates for strata below the supported ^{210}Pb horizon were extrapolated using averaged baseline sedimentation rates.

Sedimentation rate profiles (Fig. 3) are variable in nature. For Dudley, Intervale, McConnell, Wallingford, and Wheeler Ponds, sedimentation rates increase with time, with maximum values near or at the core tops, indicating possible recent disturbances in these ponds' watersheds. High and Willard Ponds display mid-core peak sedimentation, while Sessions Pond shows two distinct sedimentation peaks at ~1900 and 1970. Spring

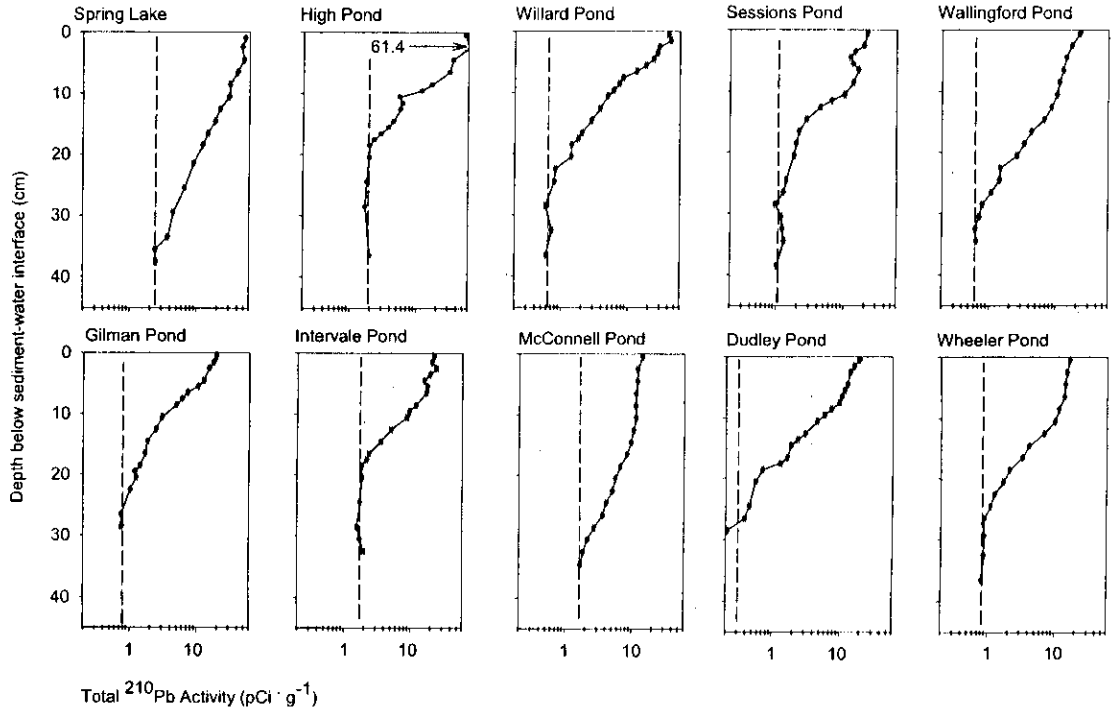

Fig. 2. Total ^{210}Pb, by depth downcore, for sediments of 10 Vermont and New Hampshire lakes.

Table 2
Supported and unsupported ^{210}Pb concentrations, densities, and fluxes, for 10 Vermont and New Hampshire lakes

	Supported ^{210}Pb, pCi g^{-1} (S.E.)	N supported samples	Cumulative unsupported ^{210}Pb, (pCi cm^{-2})	Unsupported ^{210}Pb flux, (pCi cm^{-2} yr^{-1})
Dudley	0.28 (0.07)	3	11.08	0.36
Gilman	0.75 (0.01)	2	6.77	0.22
High	1.88 (0.06)	5	14.73	0.47
Intervale	1.74 (0.06)	6	12.49	0.40
McConnell	1.68 (0.06)	1	11.32	0.36
Sessions	1.09 (0.06)	5	10.63	0.35
Spring	2.50 (0.05)	4	22.02	0.71
Wallingford	0.64 (0.02)	2	12.34	0.40
Wheeler	0.64 (0.05)	4	10.54	0.34
Willard	0.85 (0.02)	4	12.60	0.41

Lake and Gilman Pond have low, nearly flat sedimentation profiles over the period of record. Standard errors for baseline sedimentation rates for Sessions and Dudley Ponds are large.

3.2. *Hg concentrations and Hg fluxes*

Total Hg concentrations ranged from 0.06 to 0.66 μg g^{-1} (d.w.), with peak concentrations in all cases coinciding with dates of 1950 or later. Profiles of Hg concentrations in sediment reveal striking similarities (Fig. 3). For all lakes, baseline Hg concentrations of 0.06–0.21 μg g^{-1} d.w. begin to rise circa 1875, and peak between 1950 and modern times, at between 0.22 and 0.66 μg g^{-1} d.w. Most of the lakes show a decline in sediment Hg concentrations in the most recent sediments. These declines are most pronounced in Spring Lake, and High and Willard Ponds.

Mercury concentration profiles are strongly influenced by sedimentation rate, in that concentrations of elemental constituents are accentuated under periods of reduced sedimentation, and vice versa (Engstrom and Wright, 1983). Flux rates normalize this covariance, and permit comparisons across lakes. Examination of

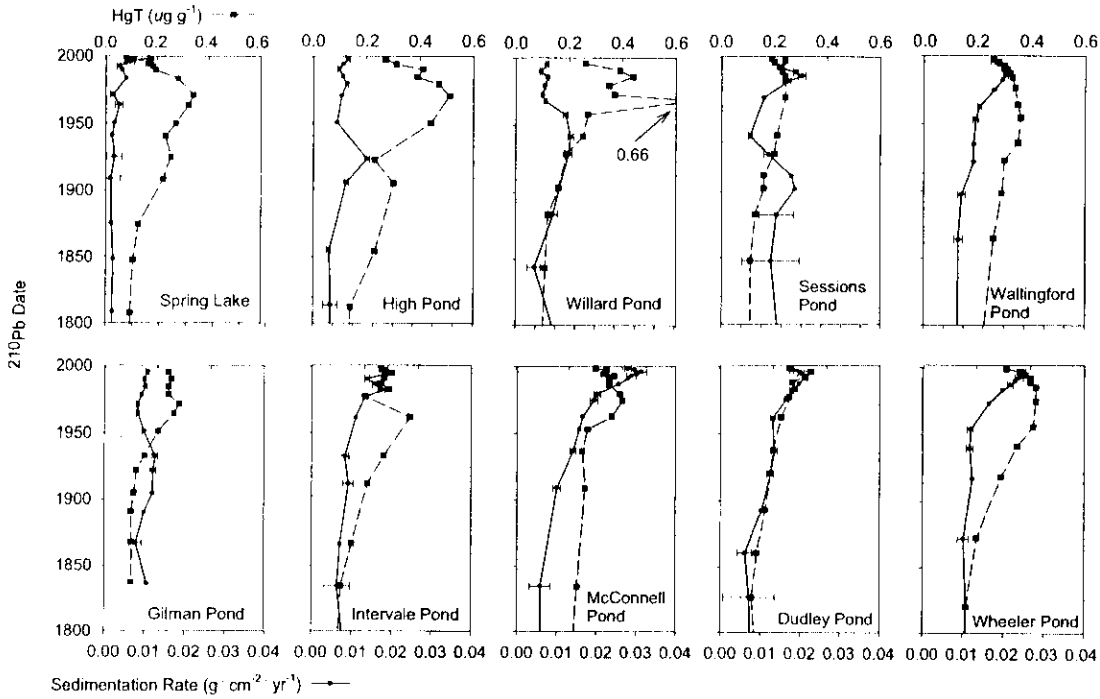

Fig. 3. Total Hg concentrations and sedimentation rates (estimated using the constant rate of supply dating model), by ^{210}Pb inferred date, for sediment cores from 10 Vermont and New Hampshire lakes. Error bars about sedimentation rates represent standard errors propagated from counting uncertainty. Rates estimated from baseline supported ^{210}Pb are shown only to the year 1800. Lakes are arrayed in order of increasing watershed:lake area.

Table 3
Total Hg fluxes, in $\mu g\,m^{-2}\,yr^{-1}$, for baseline, peak, and modern time periods, for 10 Vermont and New Hampshire lakes

	Modern (1998) Hg flux $\mu g\,m^{-2}\,yr^{-1}$	Peak flux (year of occurrence) $\mu g\,m^{-2}\,yr^{-1}$	Baseline flux (years used to estimate) $\mu g\,m^{-2}\,yr^{-1}$	Ratio of modern to baseline
Dudley	46	68 (1992)	10 (1777–1860)	4.6
Gilman	26	26 (1998)	11 (1863)	2.4
High	23	39 (1979)	5 (1693–1813)	4.6
Intervale	48	55 (1995)	7 (1626–1834)	6.9
McConnell	83	106 (1992)	13 (1777–1834)	6.4
Sessions	30	48 (1985)	14 (1766–1848)	2.1
Spring	25	41 (1963)	11(1755–1808)	2.3
Wallingford	45	66 (1987)	17 (1731–1787)	2.6
Wheeler	78	92 (1990)	16 (1715–1819)	4.9
Willard	21	50 (1968)	10 (1704–1844)	2.1
Average	42.5	52.9	11.4	3.9

baseline (pre-1850), peak, and modern (1998) fluxes (Table 3) and flux profiles (Fig. 4) reveals striking similarities. For all lakes, there was an increase in Hg fluxes, beginning by 1875. The greatest post-industrial Hg flux enhancement was observed in McConnell Pond, and the smallest, at Gilman Pond. Averaged pre-1850 Hg flux rates ranged from 5 to 17 $\mu g\,m^{-2}\,yr^{-1}$. Peak fluxes, which occurred between 1963 and modern times, varied from 26 to 106 $\mu g\,m^{-2}\,yr^{-1}$. Modern flux ratios (the ratio of modern:baseline flux) ranged from 2.1 to 6.9 (Table 3). Seven lakes displayed a continual decline in Hg flux, across four or more of the most recent core sections. This pattern has been interpreted by Engstrom and Swain (1997) as indicating a significant decline in

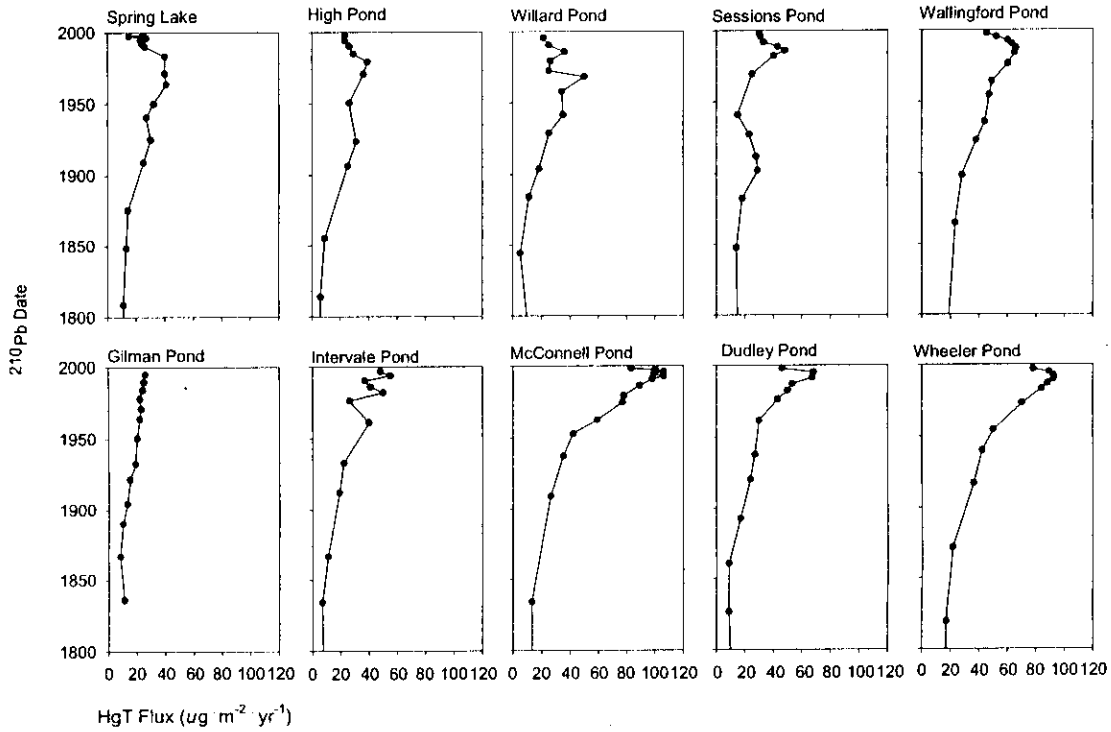

Fig. 4. Total Hg fluxes, by ^{210}Pb inferred date, to the sediments of 10 Vermont and New Hampshire lakes. Lakes are arrayed in order of increasing watershed:lake area.

atmospheric Hg loadings to several Minneapolis area lakes. Their argument that reductions in atmospheric Hg emissions from coal combustion, waste incineration, and industrial sources are responsible for reduced Hg fluxes may also apply to the present study lakes.

Our Hg fluxes and flux ratios are in excellent agreement with those reported for lakes in Northern Quebec (Lucotte et al., 1995), and the upper Midwest (Engstrom and Swain, 1997), for eight ponds and an ombrotrophic bog in Maine (Norton et al., 1997; Perry et al., 2001), for lakes in Finland, Sweden, and Western Canada (as compiled by Landers et al., 1998), and for Adirondack drainage and seepage lakes (Lorey and Driscoll, 1999). The average flux ratio (ratio of modern:baseline) for the entire lake set of 3.9 suggests that lakes across Vermont and New Hampshire have experienced a nearly four-fold increase in Hg fluxes since before 1850.

3.3. *Inferring Hg fluxes attributable to atmospheric loading*

Across a set of lakes, the relationship between the ratio of watershed:lake area and Hg flux can be used to estimate the proportion of the flux attributable to direct atmospheric contributions for any given time period (Swain et al., 1992). By this elegantly simple technique, an estimated linear function between the watershed:lake area ratio and Hg flux is backcast to a watershed:lake area ratio of one, with the corresponding flux providing an estimate of the direct atmospheric component. This assumes that evasion of Hg from lake surfaces is minimal and consistent across lakes, and that, across watersheds, a consistent proportion of the Hg transported from upstream is retained within the lake sediments. While evasion of Hg from lake surfaces has not completely been studied, the former assumption appears validated by Fitzgerald et al. (1991), who estimated that evasion accounted for no more than 10% of the Hg flux from Little Rock Lake, Wisconsin. The latter assumption is supported by Hurley et al. (2000), and Shanley et al. (2001), who have shown that the export of particulate-bound Hg varies consistently in relation to watershed DOC and sediment export, across multiple watershed scales in the upper Midwest and Northeast, respectively. However, Driscoll et al. (2001) indicate that this may not be the case for dissolved Hg moving through Adirondack systems. Thus, sediment Hg flux estimates derived by this study most accurately reflects sedimentation of particulate-bound Hg.

Fig. 5a shows linear regression models for averaged pre-1850 and modern (1998) times, across our study

lakes. Prior to 1850, there exists no significant relationship between watershed-lake area ratio and Hg flux. By contrast, for 1998, the relationship is highly significant ($F = 14.97$, $p = 0.0047$), with the variation in watershed:lake area explaining 65% of the variation in Hg flux. The estimated Hg flux attributable to direct atmospheric contributions for the pre-1850 period is $10\,\mu g\,m^{-2}\,yr^{-1}$ (S.E. = 2.0, $p = 0.001$ for Ho: atmospheric flux = 0) while the estimate for 1998 is $21\,\mu g\,m^{-2}\,yr^{-1}$ (S.E. = 7.5, $p = 0.032$ for Ho: atmospheric flux = 0). Modern atmospheric fluxes compare reasonably well to direct measurements made at a relatively high elevation Vermont site by Scherbatskoy et al. (1999). Shanley et al. (1999) used these data to estimate average annual terrestrial atmospheric fluxes of 46.3, $37.0\,\mu g\,m^{-2}\,yr^{-1}$ of which are thought to be deposited dry. These authors acknowledge that the proportion of dry-deposited Hg which is re-evaded as Hg^0 both from terrestrial and lakewater surfaces is presently unknown. Thus, our estimated modern atmospheric flux estimate of $21\,\mu g\,m^{-2}\,yr^{-1}$ is within the range of likely values for wet + dry total Hg, minus that Hg which is re-evaded from the lake surface.

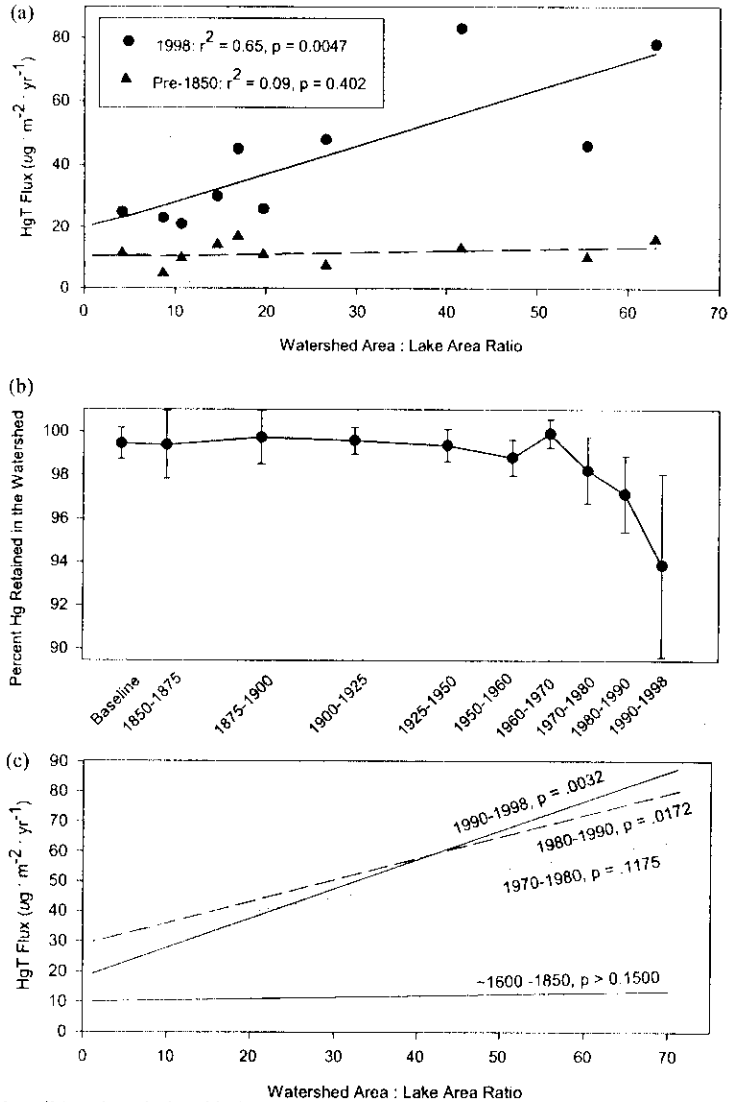

Fig. 5. (a) Linear models describing the relationship between watershed:lake area ratio, and Hg fluxes, for pre-1850 and modern (1998) time periods, for 10 Vermont and New Hampshire lakes. (b) Percent of atmospherically deposited Hg retained in these watersheds, from pre-1850 to present. (c) Linear models for the baseline period, 1970–1980, 1980–1990, and 1990–1998. Regressions are backcast to a ratio of 1:1 (shown by the dotted line), which represents the flux attributable to direct atmospheric deposition. Bars represent the standard error of the watershed retention estimates.

3.4. The role of watershed:lake area ratios in the control of Hg fluxes to lakes

Models of watershed:lake area in relation to decade-averaged fluxes from 1950 to present produce intriguing, although statistically less satisfying results. In these lakes, the relationships between watershed:lake area and Hg flux are non-significant ($p > 0.05$ for Ho: $b = 0$) except for the periods 1980–1990, and 1990–1998. During these periods, 1.2 and 0.86 $\mu g\,Hg\,m^{-2}\,yr^{-1}$ were delivered for each unit of watershed:lake area respectively; rates which are lower than the 3.27 and 1.93 $\mu g\,m^{-2}\,yr^{-1}$ reported by Engstrom et al. (1994) and Lorey and Driscoll (1999) in the Midwestern and Adirondack lakes. Thus, even though average fluxes for the 1950s and subsequent decades are significantly elevated over pre-1850 levels ($F = 4.13$, $p < 0.02$), the influence of watershed size in controlling Hg flux to this study set only becomes clear in recent years.

Viewed from the perspective of Hg retained in the watersheds (Fig. 5b), our results appear to suggest that watershed retention of atmospherically deposited Hg has declined progressively from the 1950s to the present. In reality, this trend is more likely a function of declining atmospheric Hg deposition to lake surfaces than an actual increase in export of Hg from watershed soils. A decrease in atmospheric Hg deposition should be reflected most immediately in the sediments of lakes with very small watersheds, while lakes with relatively large watersheds should continue to receive large Hg inputs (relative to direct Hg deposition to the lake surface), owing simply to a greater quantity of runoff from soils that have become saturated with anthropogenic Hg. The effect of this lag between declines in direct Hg deposition and watershed delivery is that watershed Hg loading as a percent of direct atmospheric deposition will increase, especially for lakes with large watershed:lake area ratios. This point was first proposed by Mielli (1995), and is well illustrated by the linear functions describing the relationship of Hg flux to watershed:lake area for each of the last three decades (Fig. 5c). These linear models suggest that the atmospheric component of Hg fluxes derived from larger watersheds continues to increase, even as the overall atmospheric deposition rate (e.g. model prediction at a watershed:lake area ratio of 1) appears to be declining.

Our estimate of 93.8% atmospheric Hg retention in the watersheds of the study lakes is significantly elevated over the 78% reported by both Engstrom et al. (1994) and Lorey and Driscoll (1999) for their Midwestern and Adirondack study lakes. The cause of this difference may relate either to the biogeochemistry of the watersheds, or to physical factors. Driscoll et al. (1994a, b, 1998) indicate that Hg delivery through watersheds is controlled by a variety of factors, including DOC, pH, alkalinity, and the proportion of wetlands in the watershed. Other influential factors include bedrock geology (Coker et al., 1995) and land use (Hurley et al., 2000). Alkalinity, taken here as a general indicator of DOC and pH in the watersheds, varies significantly between our study lakes, and the Adirondack and Midwestern ones ($F = 6.89$, $p = 0.005$), with log-adjusted alkalinity values significantly lower in the Adirondack lakes. However, there is no significant relationship between the estimated modern atmospheric Hg retained in the watersheds, and alkalinity ($p \geq 0.05$, $n = 3$). The geology underlying the lakes of all three lake sets, while varied, does not differ strongly, except by the presence of two highly alkaline Minnesota lakes cored by Engstrom et al. (1994). Land cover in the watersheds of all three study lake sets is predominantly forested, however, only the Adirondack lake set may have escaped the influence of deforestation in the past 150 years. No data are available regarding wetlands for either the Adirondack or Midwestern study lakes. Thus, given the available information, variation in watershed chemistry, bedrock geology, or land use cannot alone explain the high atmospheric Hg retention observed in these Vermont and New Hampshire lakes.

Morphologically, however, the study lakes are different. Watershed:lake area ratios vary significantly among these three studies ($F = 7.098$, $p = 0.004$). The present study lakes have significantly larger watershed ratios than the Midwestern sites ($p < 0.05$), which are predominantly seepage lakes. Watershed ratios for the Adirondack sites are intermediate in size, and the lakes are mixed drainage and seepage. Therefore, the larger watershed:lake area ratios in this study may explain why these Vermont and New Hampshire lakes display higher Hg retention than do the Adirondack and Midwestern study lakes. Our estimate of 93.8% Hg retained in the watersheds is in good agreement with mass-balance estimates of 92% to 94% provided by Scherbatskoy et al. (1998) for a small forested watershed adjacent to the PMRC.

4. Summary

Estimated Hg fluxes across the 10 lakes sampled in this study provide three distinct signals. First, there exists a synchronous increase in Hg fluxes across all lakes corresponding to the period 1850–1875, and Hg fluxes peak between 1955 and the present. Peak Hg fluxes are on average 3.9 times greater than average pre-1850 values, which is attributable to increased atmospheric deposition of Hg over the core record. Second, the relationship between the watershed:lake area ratio and Hg flux has become increasingly important in the past 30 years, and the modern direct atmospheric estimate of 21 $\mu g\,m^{-2}\,yr^{-1}$ is in reasonable agreement with measured atmospheric fluxes. Finally, watershed

retention of atmospherically deposited Hg, estimated at 93.8%, is elevated relative to Adirondack and Midwestern lakes, but is in good agreement with mass-balance measurements made near PMRC. A great deal of effort is presently being accorded to reduction and virtual elimination of Hg. Indeed, burning of cleaner coal and reductions in other industrial emissions may have resulted in the reduced Hg fluxes observed in this dataset in recent times. However, high watershed Hg retention, coupled with a continually increasing influence of watershed size in the downstream delivery of Hg, indicate that a significant time lag can be expected between implementation of Hg use and emission controls, and significant reductions in Hg accumulation to lake sediments. Quantification of this lag may be possible given the number of paleolimnological datasets presently available across North America, and represents a fruitful area for further analysis and inquiry.

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Methylmercury Impairs Components of the Cholinergic System in Captive Mink (*Mustela vison*)

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The effects of methylmercury (MeHg) on components of the cholinergic system were evaluated in captive mink (*Mustela vison*). Cholinergic parameters were measured in brain regions (occipital cortex, cerebellum, brain stem, basal ganglia) and blood (whole blood, plasma, serum) following an 89-day exposure to MeHg at dietary concentrations of 0, 0.1, 0.5, 1, and 2 ppm ($n = 12$ animals per treatment). There were no effects of MeHg on brain choline acetyltransferase, acetylcholine, and choline transporter. However, significantly higher densities of muscarinic cholinergic receptors, as assessed by ^{3}H-quinuclidinyl benzilate binding, were measured in the occipital cortex (30.2 and 39.0% higher in the 1 and 2 ppm groups, respectively), basal ganglia (67.5 and 69.1% higher in the 0.5 and 1 ppm groups, respectively), and brain stem (64.4% higher in the 0.5 ppm group), compared to nonexposed controls. The calculated positive relationship between MeHg exposure and muscarinic cholinergic receptor levels in this dosing study were consistent with observations in wild mink. There were no MeHg-related effects on blood cholinesterase (ChE) activity, but ChE activity was significantly higher in the occipital cortex (17.0% in the 1 ppm group) and basal ganglia (34.1% in the 0.5 ppm group), compared to nonexposed controls. The parallel increases in muscarinic cholinergic receptor levels and ChE activity following MeHg exposure highlight the autoregulatory nature of cholinergic neurotransmission. In conclusion, these laboratory data support findings from wild mink and demonstrate that ecologically relevant exposures to MeHg (i.e., 0.5 ppm in diet) have the potential to alter the cholinergic system in specific brain regions.

Key Words: mink; methylmercury; muscarinic receptor; cholinesterase; brain; wildlife; neurotoxicology.

Methylmercury (MeHg) is extremely neurotoxic, as it can readily cross the mammalian blood brain barrier and interact with protein thiols (ATSDR, 1999; Clarkson, 1997). At sub- to low-micromolar concentrations MeHg can impede essential neurophysiological processes, including microtubule formation and calcium homeostasis (Castoldi *et al.*, 2001). Although the neurotoxic effects of MeHg are mediated through multiple mechanisms, studies have shown that specific aspects of cholinergic neurotransmission are vulnerable to MeHg. *In vitro*, MeHg can inhibit the neuronal uptake of choline (Kobayashi *et al.*, 1979), activity of choline acetyltransferase (ChAT) (Dwivedi *et al.*, 1980; Kobayashi *et al.*, 1979; Omata *et al.*, 1982), and binding to the muscarinic acetylcholine (mACh) receptor (Abd-Elfattah and Shamoo, 1981; Basu *et al.*, 2005c). *In vivo*, exposure to MeHg has been linked with decreased activity of ChAT (Dwivedi *et al.*, 1980; Omata *et al.*, 1982), increased levels of mACh receptors (Coccini *et al.*, 2000), and reduced concentrations of acetylcholine (ACh) (Hrdina *et al.*, 1976; Kobayashi *et al.*, 1980). Furthermore, some of the clinical outcomes of cholinergic dysfunction (i.e., anorexia, salivation, tremors, reduced vision, seizures) (Kobayashi *et al.*, 1980; Wess, 2004) have also been observed in Hg-poisoned individuals (ATSDR, 1999; Watanabe and Satoh, 1996), thus suggesting a possible role for this neurotransmission system in the progression of MeHg toxicosis.

Mercury (Hg) is a contaminant of global concern because elemental Hg (Hg^0) can undergo long-range atmospheric transport and later be converted to MeHg, which biomagnifies through aquatic food webs (Chan *et al.*, 2003; U.S. EPA, 1997; Wiener *et al.*, 2003). Individuals at greatest risk of MeHg intoxication are obligate consumers of predatory fish. For example, ingestion of MeHg-contaminated fish by inhabitants of Minamata Bay and Niigata (Japan) circa 1950–1960 was implicated as the causative factor of Minamata disease (Watanabe and Satoh, 1996). Fish-eating wildlife are also susceptible to MeHg intoxication (Chan *et al.*, 2003; Wiener

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et al., 2003), and it should be noted that symptoms resembling Minamata disease were observed in resident animals (e.g., dogs, cats, fish) nearly 4 years before the first documented human case (Watanabe and Satoh, 1996). Controlled dosing experiments have demonstrated that piscivorous wildlife, such as mink (*Mustela vison*; Aulerich *et al.*, 1974; Wobeser *et al.*, 1976; Wren *et al.*, 1987), river otters (*Lontra canadensis*; O'Connor and Nielson, 1980), seals (*Phoca* sp.; Ronald *et al.*, 1977), and loons (*Gavia immer*; Kenow *et al.*, 2003), are sensitive to MeHg. Dietary levels as low as 1 ppm MeHg have been associated with a range of adverse outcomes at the tissue (e.g., neuronal lesions), whole-animal (e.g., effects on reproduction and neurobehavior), and possibly even population (e.g., decline in numbers) levels.

While MeHg has the potential to affect ecosystem health, nowadays fish-eating wildlife are seldom exposed to concentrations associated with overt toxic effects (i.e., >1 ppm MeHg in diet). Instead, animals are exposed to lower concentrations on a continual basis. The subtle biochemical and cellular perturbations associated with these exposures have gone largely unstudied. We recently documented that alterations in mACh receptor density can be associated with MeHg accumulation in the brains of wild mink (Basu *et al.*, 2005a). Specifically, animals with higher MeHg accumulation in brain also had greater numbers of mACh receptors. Variations in neurochemical receptors (i.e., mACh and D2 receptors; Basu *et al.*, 2005b) and enzymes (ChE and monoamine oxidase; Basu *et al.*, under review) have also been linked with MeHg exposure in North American river otters. The existence of such neurochemical changes raises numerous questions regarding the ecophysiological consequences of MeHg on wildlife populations. As disruptions to neurochemistry are known to precede structural and functional damage to the nervous system (Manzo *et al.*, 2001), alterations in brain chemistry may serve as an early warning for subsequent adverse neurological effects.

A major limitation in cross-sectional, epidemiological studies is the influence of multiple factors (e.g., co-contaminants, environmental stressors, and geographic isolation) on exposure-effect outcomes. As a result, before a causal link can be made between MeHg intake and neurochemical changes in fish-eating mammals, controlled dosing trials are required to characterize the underlying mechanisms and derive quantitative information. Wildlife are excellent models to validate the utility of neurochemical approaches, since exposure-response relationships can be assessed at multiple tiers of biological organization (i.e., laboratory experiments *in vitro*, whole-animal feeding trials, and field or ecosystem investigations), and brain tissue can be obtained for detailed analysis. Such multifaceted approaches are generally not permissible for rodents or humans. Accordingly, the present study was conducted to explore the effects of dietary MeHg on components of the cholinergic system in captive mink exposed to ecologically relevant concentrations of MeHg (i.e., 0 to 2 ppm) for 3 months.

MATERIALS AND METHODS

Chemicals. Methyl Hg chloride (>95% purity) was obtained from Alfa Aesar (Ward Hill, MA). 10-Acetyl-3,7-dihydroxyphenoxazine (Amplex Red) was purchased from Molecular Probes, Inc (Eugene, OR). ^{3}H-Acetyl CoA (200 Ci/mmol), ^{3}H-hemicholinium-3 (125 Ci/mmol), and ^{3}H-quinuclidinyl benzilate (^{3}H-QNB; 42 Ci/mmol) were obtained from NEN/Perkin Elmer (Boston, MA). All other laboratory reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Animals. Because mink are sensitive to many types of pollutants and can be studied both in captivity and in nature, they have been endorsed as excellent sentinels (U.S. EPA, 1997). Juvenile male mink (1763 ± 141 g), approximately 5 months of age at the initiation of study, were obtained from a commercial rancher and certified disease free. They were housed individually in raised wire mesh cages with nest boxes attached at the Canadian Centre for Fur Animal Research (Nova Scotia Agricultural College, Truro, NS, Canada). Mink were exposed to a natural photoperiod and temperature and had free access to water during the acclimation (~2 weeks) and exposure (~13 weeks) periods. Each animal was checked twice daily, and all aspects of this study were approved by the Nova Scotia Agricultural College Animal Care and Use Committee and carried out in strict accordance to Canadian Council on Animal Care (CCAC) guidelines.

Experimental design. Feed was prepared at the Nova Scotia Agricultural College and consisted of Atlantic herring (32%), beef tripe and liver (22%), cod (17%), barley (14%), herring oil (0.9%), and a preformulated vitamin–mineral mix. MeHg was incorporated into the diet at nominal concentrations of 0, 0.1, 0.5, 1, and 2 ppm, to reflect levels that would commonly be encountered in their natural environment (EPA, 1997). Animals were fed twice daily for a period of 89 days (August to November 2004). This length of exposure was chosen because it allows for the steady-state accumulation of Hg into tissues (Jernelov *et al.*, 1976) and is approximately 10% of a wild mink's lifespan (Lariviere, 1999).

At the termination of the study, each animal was anesthetized with an im injection of xylazine (2 mg/kg bw) and ketamine hydrochloride (25 mg/kg bw). Blood was drawn via cardiac puncture, and the animals were sacrificed with an overdose of pentobarbital (105.6 mg/kg bw ic). Blood samples were kept on ice for approximately 4–6 h before they were separated into plasma and serum as described by Stamler *et al.* (2005), and then stored at −80°C. The entire brain was extracted from the skull, and specific regions (occipital cortex, cerebellum, brain stem, and basal ganglia) were dissected from the right hemisphere and stored at −80°C. These regions were studied because their structure and/or function have previously been shown to be affected by MeHg (ATSDR, 1999; Clarkson, 1997). Total Hg was measured in the feed and the tissues (brain and blood) according to Evans *et al.* (2000).

Neurochemical assays. Tissues were prepared as described by Stamler *et al.* (2005) with minor modifications. All brain samples were homogenized for 30 s in cold Na/K buffer (50 mM NaH_2PO_4, 5 mM KCl, 120 mM NaCl, pH 7.4). For binding assays, cellular membranes were isolated by centrifuging the homogenate at 32,500 × *g* for 15 min at 4°C. The resulting pellet was washed twice under the same conditions, and the final pellet was resuspended in Na/K buffer. For ChAT, ACh, and ChE analyses, Triton-X (final concentration = 0.1% w/v) was added to the homogenate followed by a 20-s sonication. Protein concentration was determined using the Bradford protocol. Samples were stored at −80°C prior to analysis.

ChAT activity. The activity of ChAT was determined by the method of Fonnum (1975) with modifications. The assay was carried out by incubating samples (10 µg protein) in 50 mM Na/K buffer containing 10 mM EDTA, 100 µM eserine, 100 mM choline chloride, and 0.2 µCi ^{3}H-acetyl CoA for 30 min at 37°C. The reaction was terminated by adding an equal volume (200 µl) of 1.5% tetraphenyl boron, followed by vigorous shaking and centrifugation at 3750 × *g* (10 min, 25°C) to separate the phases. The activity of ChAT was determined by measuring ACh in the organic layer, and the results were expressed as fmol ACh formed/min/µg protein.

ACh concentration. Concentrations of ACh were quantified using a commercially available kit (Molecular Probes Inc., Eugene, OR) with minor modifications. Samples (10 μg protein) were incubated in Na/K buffer including 100 μM Amplex Red, 200 mU horseradish peroxidase, 20 mU choline oxidase, and 5 U acetylcholinesterase (AChE). Formation of the assay end-product, resorufin, from ACh was determined following a three-step enzymatic reaction catalyzed by AChE, choline oxidase, and hydrogen peroxidase. Fluorescence of resorufin ($\lambda_{ex} = 540$, $\lambda_{em} = 590$) was monitored in a microplate fluorometer (FLUOstar Optima, BMG Laboratories, Offenburg, Germany) following a 30 min incubation period. The concentration of ACh was determined from a standard curve (0–2 μM ACh chloride) and expressed as nM ACh per mg protein.

mACh receptor binding assay. Binding to the mACh receptor was performed in a 96-well 1.0 μM GF/B glass filter system (Millipore, Boston, MA) as previously described (Stamler *et al.*, 2005). Approximately 20 μg of membrane preparation in Na/K buffer was incubated with 1 nM ^{3}H-QNB, a concentration that is indicative of mACh receptor density in mink (Stamler *et al.*, 2005). Following a 60-min incubation period under gentle agitation, the binding assay was terminated by vacuum filtration, and the filters were washed three times with Na/K buffer and then allowed to soak overnight in scintillation cocktail. The radioactivity retained by the filters was quantified by a liquid scintillation counter (Beckman LS3801, Fullerton, CA) with approximately 60% counting efficiency. Specific binding was defined as the difference in ^{3}H-QNB bound in the presence and absence of 100 μM atropine sulphate.

To compare the relationship between brain Hg and mACh receptor levels from the current study (i.e., data for four discrete brain regions) with a prior cross-sectional field experiment on wild mink (i.e., data for whole brains) (Basu *et al.*, 2005a), receptor data from the current study were normalized to provide a relative measure of possible levels in the whole brain. This was achieved by taking into consideration that the average weight of the whole mink brain was 10.88 ± 0.81 g, and that approximate weights of the individual regions were: occipital cortex (1.0 g), cerebellum (1.2 g), brain stem (0.8 g), and basal ganglia (0.3 g). The weights of these regions were estimated by measuring the amount of tissue extracted during the necropsy, and the values compare favorably to rodents (Scheuhammer and Cherian, 1982). It should be noted that assumptions in this approach are that Hg levels are uniformly distributed throughout the mink brain, and that MeHg-related changes in mACh receptor levels are localized only to the specific brain regions we explored.

ChE activity. The activity of ChE in brain and blood samples was determined according to protocols described by Stamler *et al.* (2005). The assay was carried out by incubating samples (0.1 μg of brain protein, or 1:5151 diluted whole blood, plasma, or serum) in Na/K buffer containing 100 μM Amplex Red, 200 mU horseradish peroxidase, 20 mU choline oxidase, and 100 μM ACh chloride. After a 30-min incubation period, the reaction end-product, resorufin, was detected as described in the section *ACh concentration.* The specific activity of ChE was expressed as nmol of resorufin formed per min per protein (μg) or volume (μl).

Choline transporter. Binding to the high-affinity choline transporter was based on the method of Vickroy *et al.* (1984) and modified for a 96-well microplate filtration system. Approximately 20 μg of membrane preparation was preincubated for 30 min in Na/K buffer in filter plates (1.0 μM GF/B glass filters, Millipore, Boston, MA) that were presoaked with 0.1% polyethylenimine. Samples were then incubated with 2 nM ^{3}H-hemicholinium-3 for 20 min at 25°C under gentle agitation. The binding assay was terminated, and radioactivity was quantified according to the methods described in the section *mACh receptor binding assay.* Specific binding was defined as the difference in ^{3}H-hemicholinium-3 bound in the presence and absence of unlabelled hemicholinium-3 (10 μM).

Statistical analyses. For quality control, method blanks and positive controls were included in all biochemical assays. An internal standard was created by pooling brain tissues from five untreated mink, and this sample was used to calculate inter- and intra-assay variation. All samples were assayed in triplicate.

A p value less than or equal to 0.05 was considered statistically significant in all analyses. All statistical analyses were performed using SPSS version 11.5 (Chicago, IL). Data are represented as means ± SD. The difference in the nominal and actual concentrations of Hg in the diet was assessed by a Mann-Whitney U test. Concentrations of MeHg in blood and brain were log-transformed to satisfy the assumptions of parametric statistics. One-way analysis of variance (ANOVAs) was used to determine the effects of dietary MeHg on the cholinergic parameters and Hg burden. When significant differences were found, post-hoc comparisons were performed with Tukey's HSD. Pearson correlations were used to determine the association between neurochemical parameters in components of blood and brain regions. The slopes of the regression plots relating brain Hg with mACh receptor levels from the laboratory and field study were compared using a general linear model.

RESULTS

No mortalities or obvious changes to animal behavior were evident during the trial. The background level of Hg in the control diet (i.e., 0 ppm MeHg) was 22 ± 7 ng/g, and the measured concentrations of dietary MeHg in the treatments were not significantly different from the nominal values. Given that mean daily feed intake among all treatments was 313 ± 63 g feed per animal, dietary exposures ranged from 3.3 to 267.8 μg/kg b.w./day ($F_{4,20} = 246.2, p < 0.001$) (Table 1). A significant exposure-dependent increase in total Hg was measured in blood ($F_{4,55} = 7.9$, $p < 0.001$) and brain ($F_{4,55} = 597.5$, $p < 0.001$) (Table 1). There were no significant MeHg-related changes in feed intake, whole body, and brain weight.

Cholinergic Measurements

The inter- and intra-assay variation in all assays was less than 15%, except for the measurement of choline transporter (intra-assay CV = 23.3%). There were no MeHg-related effects on ChAT activity, ACh concentrations, or choline transporter in different regions of the brain (data not shown). However, significant MeHg-dependent increases in mACh receptor levels were measured in all brain regions studied except for the cerebellum (Fig. 1). ^{3}H-QNB binding in the occipital cortex was significantly ($F_{4,55} = 3.8$, $p < 0.01$) higher in the 1 and 2 ppm dietary groups (30.2 and 39.0%, respectively), compared to controls (Fig. 1A). In the basal ganglia, exposure to 0.5 and 1 ppm MeHg resulted in significantly ($F_{4,55} = 2.8$, $p < 0.05$)

TABLE 1
Concentrations of Total Hg in the Diet and Tissues

MeHg (ppm) in diet, nominal	Intake (μg/kg bw/day)	Whole blood (ppm w.w.)	Brain (ppm d.w.)
0	3.3 ± 0.6^a	0.14 ± 0.27^a	0.41 ± 0.09^a
0.1	17.5 ± 1.7^b	0.60 ± 0.81^b	1.50 ± 0.34^b
0.5	77.4 ± 7.6^c	0.68 ± 0.72^b	4.09 ± 0.98^c
1	162.5 ± 23.5^d	0.68 ± 0.47^b	7.13 ± 0.94^d
2	267.8 ± 24.9^e	1.42 ± 0.80^b	15.38 ± 3.92^e

Note. Numbers represent means ± SD, and letters represent significant differences within columns.

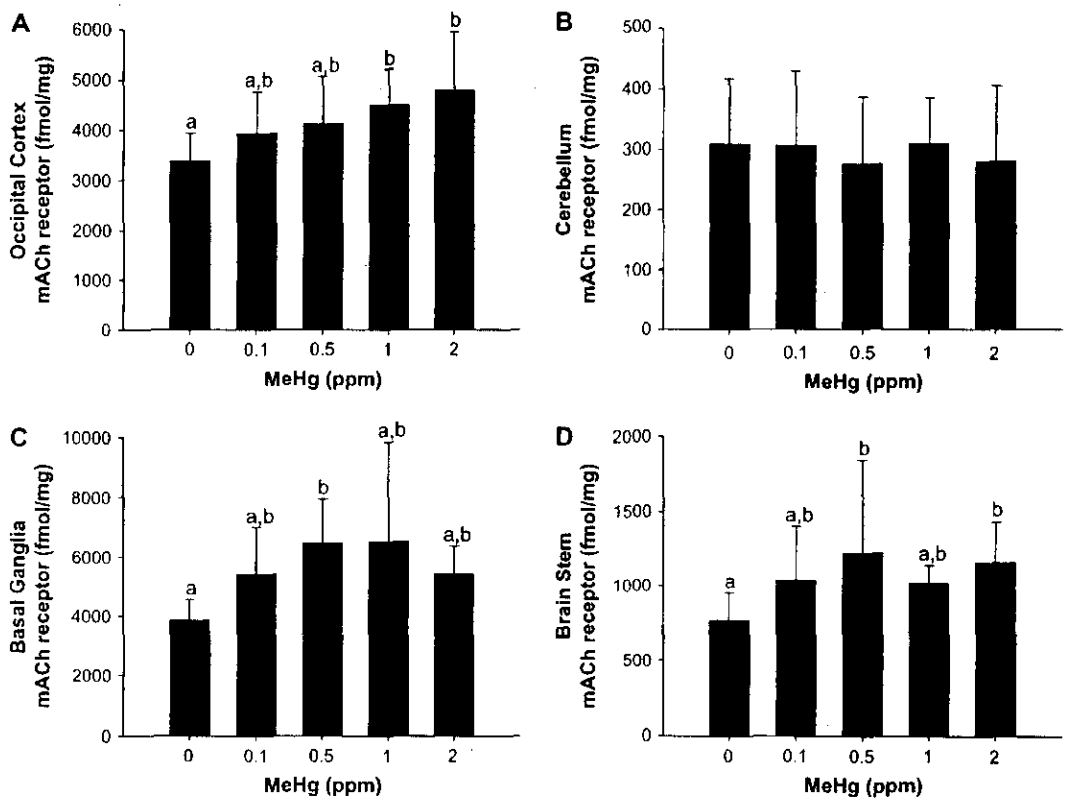

FIG. 1. Muscarinic acetylcholine (mACh) receptor binding in discrete brain regions (A—occipital cortex; B—cerebellum; C—basal ganglia; D—brain stem) of captive mink (*Mustela vison*) following an 89-day exposure to dietary methylmercury (MeHg). Bars represent means (±standard deviation) from 12 animals. Letters denote significant ($p < 0.05$) differences among the treatments following one-way analysis of variance (ANOVA).

higher ^{3}H-QNB binding (67.4 and 69.1%, respectively) compared to controls (Fig. 1C). In the brain stem, mean ^{3}H-QNB binding was significantly ($F_{4,55} = 2.9$, $p < 0.05$) higher (64.4%) in the 0.5 ppm dietary group relative to controls (Fig. 1D).

For ChE activity, MeHg-related increases were measured in the occipital cortex and basal ganglia (Fig. 2). The activity of ChE in the occipital cortex was significantly ($F_{4,55} = 3.2$, $p < 0.05$) higher in the 1 ppm MeHg group, compared to the 0 and 2 ppm dietary groups, by 17.0 and 18.4%, respectively (Fig. 2A). In the basal ganglia, ChE activity was significantly ($F_{4,55} = 3.1$, $p < 0.05$) higher by 34.1% in the 0.5 ppm group compared to nonexposed controls (Fig. 2C).

In the components of blood tested (i.e., whole blood, plasma, serum) there were no effects of MeHg on the activity of ChE (data not shown). However, there were significant correlations between enzyme activity in plasma and brain stem ($r = 0.516$, $p < 0.0001$), plasma and occipital cortex ($r = 0.250$, $p < 0.05$), and whole blood and basal ganglia ($r = -0.404$, $p < 0.001$).

Comparison of mACh Receptor Levels between Lab and Field Studies

The relationship between brain Hg and mACh receptor levels were compared between the current laboratory study and a previous field experiment (Fig. 3). By normalizing the receptor data from the current dataset (i.e., studied in four discrete brain regions) to reflect values in the entire brain, the densities of mACh receptor (dependent variable) could be related to Hg (independent variable) in whole brains according to the following equation: $y = 129.8\text{Ln}(x) + 1507.5$ ($r^2 = 0.176$, $p < 0.001$). This exposure-response relationship is similar to the field study ($y = 118.8\text{Ln}(x) + 629.4$; $r^2 = 0.299$, $p < 0.0001$), as no statistically significant differences were obtained between the slopes of the two regression plots.

DISCUSSION

Studies in wild (Basu *et al.*, 2005a,b, under review) and laboratory (Coccini *et al.*, 2000; Dwivedi *et al.*, 1980; Hrdina *et al.*, 1976; Kobayashi *et al.*, 1980; Omata *et al.*, 1982) animals have shown that MeHg can alter components of the cholinergic system. The effects on this system are nonspecific and are caused by the interactions of MeHg with sulfhydryl-containing proteins, which are ubiquitous in all cells. Thus, to properly evaluate the effect of MeHg on cholinergic neurotransmission, we have systematically investigated the key biochemical parameters in this pathway. While no changes to brain

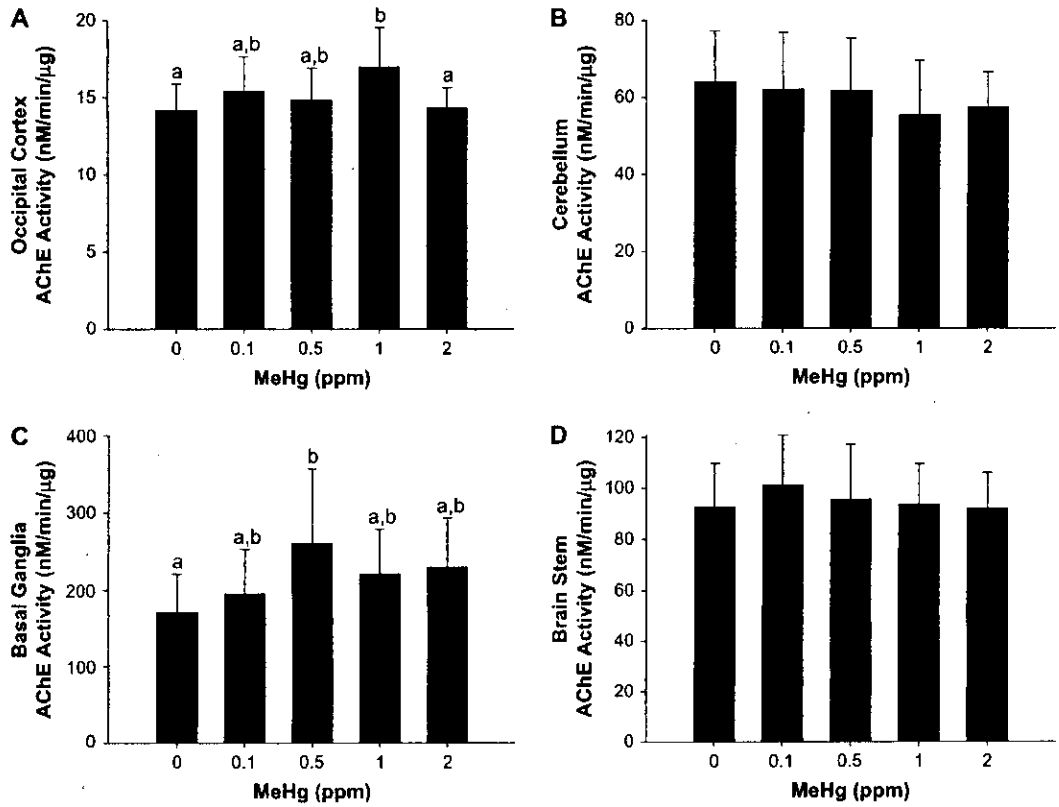

FIG. 2. Activity of cholinesterase (ChE) in discrete brain regions (A—occipital cortex; B—cerebellum; C—basal ganglia; D—brain stem) of captive mink (*Mustela vison*) following an 89-day exposure to dietary methylmercury (MeHg). Bars represent means (±standard deviation) from 12 animals. Letters denote significant ($p < 0.05$) differences among the treatments following one-way analysis of variance (ANOVA).

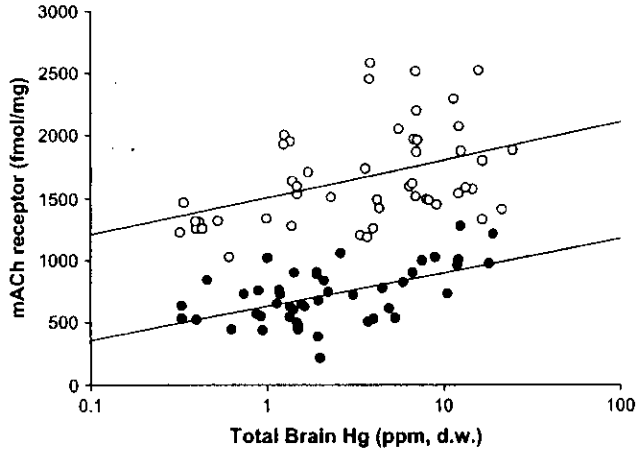

FIG. 3. Relationship between concentrations of mercury (Hg) and levels of muscarinic acetylcholine (mACh) receptors in the brain tissues of captive (open circles—○) and wild (closed circles—●) mink. The data from the laboratory were normalized from receptor levels measured in four discrete brain regions (i.e., occipital cortex, cerebellum, basal ganglia, and brain stem). The data from the field were obtained from a previous publication (Basu *et al.*, 2005a). Lines of best fit were calculated with linear regression modeling.

ChAT, ACh, or choline transporter were found, significant increases of mACh receptor levels (Fig. 1) and activity of ChE (Fig. 2) were related to dietary MeHg intake. The mechanisms underlying these observations can be attributed to the tightly regulated and homeostatic controlled cholinergic system.

Exposure of mammals to MeHg results in a net decrease of cholinergic signals through the central nervous system. *In vitro*, MeHg can inhibit the activity of ChAT (i.e., decreased synthesis of ACh; Dwivedi *et al.*, 1980; Kobayashi *et al.*, 1979; Omata *et al.*, 1982), ligand binding to the mACh receptor (i.e., reduced signal transduction; Abd-Elfattah and Shamoo, 1981; Basu *et al.*, 2005c), and reuptake of choline (i.e., reduced ACh turnover; Kobayashi *et al.*, 1979). These results are supported *in vivo*, as MeHg can reduce ChAT activity (Dwivedi *et al.*, 1980; Omata *et al.*, 1982) and ACh content (Hrdina *et al.*, 1976; Kobayashi *et al.*, 1980). Despite these data, we did not measure any changes in ChAT activity, ACh concentration, or levels of choline transporter. Prior documentation of ChAT inhibition was obtained from acute, high-dose experiments on rats (i.e., 2–10 mg/kg bw/day for 1–4 weeks; Dwivedi *et al.*, 1980; Omata *et al.*, 1982). Conversely, our study was designed to mimic an ecologically relevant scenario (i.e., <0.27 mg/kg bw/day for 12.7 weeks). Perhaps no effects on ChAT were

found because the burden of MeHg in the brain was not sufficiently high to affect enzyme activity, or the animals adapted to the continuous exposure. While MeHg can inhibit the reuptake of choline into the neuron *in vitro* (Kobayashi *et al.*, 1979), to our knowledge, MeHg-induced changes in the levels of this transport protein have not been observed *in vivo*. Interestingly, the blockage of choline uptake in rats by MeHg or hemicholinium (a potent inhibitor of choline uptake) results in similar biochemical (i.e., reduced ACh content and turnover) and behavioral (e.g., tremors, staggered gait, and depression) outcomes (Kobayashi *et al.*, 1980). It was not possible to postulate whether the neuronal content of ACh was affected by MeHg in our study. The accurate estimation of this neurotransmitter requires immediate decapitation or microwave irradiation, as ACh is rapidly hydrolyzed by ChE (turnover rate = 150 μsec; Goldberg and Hanin, 1976). Neither method was an option in the present study because of animal care protocols. Because chronic exposures to low levels of MeHg can be related to decreased ACh content in rat brains (Hrdina *et al.*, 1976), carefully designed studies will be required to reconcile this outcome in wildlife.

The key finding of this study was that the variation in mACh receptor levels could be related to the intake of MeHg. The mACh receptor belongs to a highly conserved class of membrane-spanning proteins that transduce intracellular signals through a G-protein (Wess, 2004). MeHg can directly affect this receptor by inhibiting ligand binding (Abd-Elfattah and Shamoo, 1981). For example, the calculated IC50 in the cerebral cortex of mink (5.5 μM MeHg) was within four-fold of values in humans and rodents (Basu *et al.*, 2005c). Because Hg can impair ligand binding, up-regulation of the mACh receptor is possibly a compensatory mechanism to ensure homeostasis in cholinergic transmission. Coccini *et al.* (2000) also found increased mACh receptors (20 to 44% over controls) in adult female Sprague-Dawley rats exposed to MeHg (0.5 mg/kg bw/day) for 16 days. However, these changes were measured 14 days following the termination of treatments and were localized to the hippocampus and cerebellum. No MeHg-related alterations in mACh receptor levels were found in the cerebral cortex. Whether there is a species difference in the susceptibility among different brain regions requires further study. A similar positive correlation was observed between concentrations of brain Hg and mACh receptor levels in wild mink collected from three study sites across Canada in a cross-sectional study (Basu *et al.*, 2005a). More importantly, there was no significant difference in the slopes of the regression curves obtained from the current laboratory study and the previous field investigation (Fig. 3). Because consonant exposure-response relationships infer a common mode of action, these findings collectively suggest that exposure to ecologically relevant concentrations of MeHg can be related to higher levels of mACh receptors in populations of wild mink. The existence of this phenomenon in natural populations is further supported by considering that Hg-related changes to the cholinergic pathway have also been observed in feral river otters (Basu *et al.*, 2005b, under review).

MeHg-related increases of ChE activity were measured in the occipital cortex and basal ganglia (Fig. 2). *In vitro*, MeHg does not inhibit ChE activity in mink (N. Basu, unpublished data) or rodents (Kobayashi *et al.*, 1979, 1980). Therefore, changes in enzyme activity are likely secondary responses resulting from variations in mACh receptor levels. Several studies have shown that pharmacological agents and environmental pollutants can induce unidirectional changes to the mACh receptor and ChE activity. For example, decreases in both ChE activity and mACh receptors are common outcomes in animals poisoned by organophosphates (Costa *et al.*, 1982). In nature, a positive correlation has been calculated between mACh receptor levels and ChE activity in wild birds exposed to pesticides (Burn and Leighton, 1996) and in wild river otters exposed to Hg (Basu *et al.*, under review). These examples highlight the autoregulatory nature of the cholinergic system during periods of toxicant stress.

MeHg causes discrete lesions to the calcarine region of the occipital cortex and to the granule layer of the cerebellum in wildlife (Wobeser *et al.*, 1976; O'Conner and Nielson, 1981), rodents, and humans (Watanabe and Satoh, 1996), and these homologous responses in different mammalian species suggest a common mode of action. While neurochemical changes would be expected in the two aforementioned brain regions, alterations in mACh receptors and ChE activity were measured only in the occipital cortex (Figs. 1A and 2A). The lack of observable response in the cerebellum (Figs. 1B and 2B) is likely due to the scarcity of cholinergic neurons in this brain region, although further research is required because prior studies have found MeHg-related effects on the mACh receptor in this region (Basu *et al.*, 2005c; Coccini *et al.*, 2000). Changes in cholinergic parameters were also measured in the basal ganglia and brain stem, and some clinical outcomes of MeHg poisoning have been linked to functional impairments in these brain structures. For example, MeHg-induced hand tremors (Fawer *et al.*, 1983) and auditory-evoked potentials (Murata *et al.*, 1999) can be related to damage to the basal ganglia and brain stem, respectively.

Monitoring neurochemistry is a novel approach to predict and/or detect early nervous system dysfunction, because alterations in cellular biochemistry are known to precede permanent tissue damage (Manzo *et al.*, 2001). In the current study, a continuum of MeHg-related neurological effects was observed whereby exposure of mink to the lowest treatment (i.e., 0.1 ppm) resulted in significant uptakes of Hg into brain (Table 1), but no significant changes in neurochemistry. With increasing exposure to dietary MeHg, neurochemical effects became evident in animals exposed to 0.5 ppm MeHg (Figs. 1 and 2). Prior studies have shown the emergence of neuropathology in mink exposed to 1 ppm dietary MeHg (Wobeser *et al.*, 1976; Wren *et al.*, 1987). It is interesting to note that alterations in neurochemistry often subsided in the highest exposure groups (e.g., mACh receptor levels in the basal ganglia and brain stem,

Figs. 1C and 1D, respectively), and this attenuation may be related to the animal's inability to maintain cellular homeostasis once cytotoxicity becomes imminent.

In summary, the present study demonstrated that MeHg can affect certain parameters of the cholinergic system in captive mink. Specifically, MeHg-related increases in mACh receptor levels (Fig. 1) and ChE activity (Fig. 2) were measured in discrete regions of the brain. Furthermore, the neurochemical changes occurred at a MeHg exposure level (i.e., 0.5 ppm) that is below dietary concentrations (i.e., ~1 ppm; Wobeser *et al.*, 1976; Wren *et al.*, 1987) known to cause structural and functional damage. The results from this laboratory study corroborate ecological findings and suggest that high MeHg exposure is associated with increased mACh receptor density in mink (Fig. 3). Collectively, the emerging evidence from the laboratory and the field demonstrate that ecologically relevant concentrations of MeHg can affect cholinergic neurotransmission in fish-eating wildlife.

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CHAPTER 16

Ecotoxicology of Mercury

James G. Wiener, David P. Krabbenhoft, Gary H. Heinz, and Anton M. Scheuhammer

CONTENTS

16.1 INTRODUCTION

This chapter describes selected aspects of the behavior of mercury in the environment and examines the ecotoxicology of this highly toxic metal. The widespread geographic extent and

adverse consequences of mercury pollution continue to prompt considerable scientific investigation. Furthermore, the environmental sources, biogeochemistry, transformations, transport, fate, and effects of mercury in the environment are subjects of frequent symposia, workshops and a large, steadily expanding body of scientific literature. We characterize the environmental mercury problem, critically review the ecotoxicology of mercury, and describe the consequences of methylmercury contamination of food webs. We discuss processes and factors that influence exposure to methylmercury, the highly neurotoxic form that readily accumulates in exposed organisms and can biomagnify in aquatic and terrestrial food webs to concentrations that can adversely affect organisms in upper trophic levels, including humans. Emphasis is given to aquatic food webs, where the problem of methylmercury contamination is greatest.[1] When available, recent reviews have been cited for readers interested in more detailed coverage.

Concerns about environmental mercury pollution and contamination of aquatic food webs stem largely from the human health risks of dietary exposure to methylmercury, the dominant form of mercury in the edible flesh of fish and aquatic mammals.[2–4] The human health risks associated with mercury in surface waters and aquatic biological resources are not reviewed here but have been critically examined in several case studies and recent reviews.[5–16] Nonetheless, our discussion of processes and factors affecting exposure of fish and wildlife to methylmercury is directly relevant to the issue of human exposure to methylmercury, which results largely from consumption of fish, shellfish, and aquatic mammals and birds.[10,12,17]

16.2 EVOLUTION OF THE ENVIRONMENTAL MERCURY PROBLEM

Humans have been using mercury for more than 2000 years for a wide variety of applications,[18,19] and centuries of emissions and reemissions of anthropogenic mercury have caused widespread environmental contamination over large regions of the globe.[20,21] Cinnabar, $HgS_{(S)}$, the principal mercury ore, was used as a red pigment long before the process for refining mercury ore to recover elemental mercury, Hg^0, was discovered. Since the advent of refining cinnabar, five mining areas have dominated the historical global production of elemental mercury: the Almadén district in Spain, the Idrija district in Slovenia, the Monte Amiata district in Italy, the Huancavelica district in Peru, and the state of California in the United States.[18,22] At Almadén, Spain, mercury was first mined about 430 B.C.,[23] and during the next 25 centuries the Almadén mines produced more than 280,000 metric tons of the estimated total global production of about 800,000 tons.[22] The mining and smelting of cinnabar and other mercury ores have caused substantial contamination of air, soil, water, biota, and sediment in the vicinity of such operations, and mercury-containing wastes at mining and smelting sites continue to emit mercury, including methylmercury, to the environment for decades or centuries after operations cease.[22,24–30]

From 1550 to 1930 an estimated 260,000 tons or more of mercury were released globally from mining operations that used the mercury-amalgamation process to recover gold and silver.[31] In the United States, gold mining was the primary use of mercury during the latter half of the 1800s, and the demand created by gold and silver mining stimulated mining for mercury as well.[18,32] The mining of mercury deposits (primarily cinnabar) along 400 km of the Coast Range of California, for example, was stimulated by the California gold rush in the mid-1800s.[28,33,34]

Gold or silver was mined throughout much of North America, and large quantities of mercury were used for precious-metal mining in California, Nevada, and South Dakota.[31,34] Contaminated tailings and alluvium originating from mining sites are consequently widespread in North America and elsewhere.[29,31,35–37] Emissions of mercury from contaminated mine tailings and lands include volatilization of Hg^0 to the atmosphere, aqueous dissolution by infiltrating water and entrainment with stream flow, and physical erosion and downstream transport of mercury-enriched geologic materials.[37–42] Contaminated tailings can remain a source of mercury emissions for decades or centuries after mining operations have ceased.[31,37] In some drainage basins, exemplified by the

Carson River (Nevada), contaminated sediment originating from historic mining sites has been transported, deposited, and redistributed far downstream, causing persistent contamination of stream and river channels, river banks, floodplains, and reservoirs along extensive reaches of the watershed.[33,35-38,40,41,43,44] The natural burial of such mercury-contaminated deposits by more recent, "clean" sediments may mitigate these settings only temporarily, given that large floods can reexpose the underlying, contaminated deposits.[36]

Since the early 1970s there has been a resurgence of gold-mining operations that use the mercury-amalgamation process, particularly in South America, Southeast Asia, China, and parts of Africa.[31,37,45-47] These ongoing mining activities, which seem to be stimulated partly by economic recession,[46] are widely dispersed in hundreds to thousands of operations — often small and in remote areas — involving millions of people worldwide.[31,45,46] Total emissions from these operations are now and could remain a globally significant source of new anthropogenic mercury for decades.[31,37,39,45] Recent emissions to the global environment from this "new gold rush" may total as much as 460 metric tons per year (about 10% of annual, anthropogenic global emissions),[48] with roughly two thirds of this total emitted to the atmosphere and one third emitted to land or water.[31] In Brazil, gold mining has become the major source of anthropogenic mercury emissions.[45]

Mercury also has a long history of usage in industrial applications, particularly in chlor-alkali plants and pulp and paper mills, and pollution from these sources has been well documented in recent decades.[21,25,49,50] The most publicized industrial releases occurred in Minamata and Niigata, Japan, in the 1950s and 1960s, when many humans were poisoned by methylmercury after eating fish that were highly contaminated by mercury from direct industrial sources.[5,8] These tragedies focused global attention on environmental mercury pollution[51] and prompted efforts, beginning around 1970 in the United States, Canada, and many other industrialized countries, to identify industrial sources of mercury pollution and to reduce intentional discharges of mercury into surface waters.[25,45] As a result, mercury levels in fish and sediments in such industrially affected waters typically declined in subsequent years and decades.[25,52-60] In many cases, the concentrations of mercury in fish decreased by 50% or more during the first decade after discharges were reduced, and the rate of decrease in concentration then slowed considerably, or concentrations leveled off, to values that were elevated relative to lesser contaminated waters nearby.[25,52,53,55,58,61] At some mercury-contaminated sites, however, the decline in concentrations of mercury in fish has been slow or delayed in the affected aquatic ecosystem.[25,62,63]

In industrially polluted Clay Lake, Ontario, mercury concentrations in gamefish have declined from peak levels but remained substantially above the Canadian mercury limit of 0.5 μg/g wet weight nearly three decades after operations ceased at the industrial source, a chlor-alkali plant near Dryden that operated from 1962 to 1970.[52,64] Mercury concentrations in axial muscle of 50-cm walleye (*Stizostedion vitreum*) from Clay Lake decreased rapidly after operations ceased at the chlor-alkali plant — from about 15 μg/g wet weight in 1970 to about 7.5 μg/g in 1972 — and then declined gradually to about 3.5 μg/g in 1983.[52] Concentrations apparently declined little during the next 15 years, given that total mercury averaged 2.7 μg/g in a sample of 14 walleyes (mean fork length, 53 cm) taken from Clay Lake in 1997 and 1998.[64] Persistent problems with methylmercury contamination of aquatic biota at historically contaminated sites may result from continuing, unintended emissions of mercury from the source area, from recycling and methylation of the mercury present in contaminated sediments, from temporal increases in the bioavailability of mercury or in the habitability of highly contaminated zones within the ecosystem, from changes in food-web structure, from atmospheric deposition of mercury from other sources, or from a combination of these and other factors.[25,50,62,65-69] Indeed, the physical and chemical properties that made mercury so useful in industrial applications (e.g., liquid state at ambient temperature, high volatility, and ease of reduction) also make this metal very difficult to contain and recover from the environment.[25]

The growing awareness of the hazards of mercury exposure led to widespread discontinuation or phased reductions in usage of the metal in a variety of applications and consumer goods beginning

in the late 1960s.[18,19,29] For example, the use of mercurial fungicides in seed grain, which began in the 1940s, had severe consequences for humans and wildlife. Thousands of humans were poisoned, and hundreds died, when methylmercury-treated grains were eaten (rather than planted) by Iraqi farmers and their families.[6,7,9] Incidents of high mortality of wild birds were reported after planting of seeds treated with alkylmercury compounds,[70] and both seed-eating birds and their predators were poisoned.[71] The use of mercury compounds as seed dressings was decreased or banned in Sweden, Canada, and the United States in the 1960s and 1970s.

Mining of mercury decreased abruptly in response to rapidly declining demand and prices. Mercury production in the United States, for example, had peaked in 1877 at more than 2700 metric tons per year, and as recently as 1969 there were more than 100 active mercury mines in the country.[18] The mercury-mining industry in the United States collapsed in the early 1970s. Fewer than ten mines remained in production in late 1976, and the last mine in the country to produce mercury as its principal product closed in November 1990.[18]

In the late 1970s and 1980s, concentrations of mercury exceeding 0.5 or 1.0 μg/g wet weight — sufficient to prompt fish-consumption advisories — were reported in predatory fishes from aquatic ecosystems lacking substantive, on-site anthropogenic or geologic sources of mercury.[72–76] Subsequent investigations have shown that in certain aquatic systems concentrations of methylmercury in aquatic invertebrates, fish, and piscivorous wildlife are commonly elevated — a situation frequently reported for humic and low-alkalinity lakes (including low-pH lakes),[77–83] newly flooded reservoirs,[84–88] and wetlands or wetland-influenced ecosystems.[67,89–91] Many such environments can be characterized as lightly contaminated systems in which the amount of inorganic Hg(II) being converted to methylmercury is sufficient to contaminate food webs supporting production of fish and wildlife.[92–100]

Reliable records of temporal trends in mercury deposition can be obtained by analyses of dated cores of depositional sediments from lakes or reservoirs, of peat from ombrotrophic bogs, and, in some cases, of glacial ice.[20,59,101–106] At a site in northwestern Spain about 600 km northwest of the Almadén mines, substantive anthropogenic emissions of mercury to the atmosphere are reflected in peat deposited more than 1000 years ago in a core from an ombrotrophic bog.[23] The oldest anthropogenic mercury in this core was deposited about 2500 years ago, coinciding with the start of mining at Almadén and accounting for about 10 to 15% of the total mercury deposited in peat at that time.[23] In remote and semiremote areas of North America, Greenland, and Scotland that lack on-site sources of anthropogenic mercury, the rate of mercury accumulation in many lacustrine sediments has increased by a factor of 2 to 4 since the mid-1800s or early 1900s, based on analyses of sediment and peat cores.[101,102,104,107–110] Moreover, some cores from semiremote sites show evidence of recent declines in atmospheric mercury deposition associated with decreasing regional emissions of anthropogenic mercury into the environment.[102,103,106,109] Much of the mercury deposited onto terrestrial catchments is stored in soils, and the sediments in lakes that receive substantial inputs of mercury from their catchments may be slow to reflect declines in rates of atmospheric deposition of mercury.[110]

Many remote and semiremote ecosystems are contaminated with anthropogenic mercury deposited after long-range atmospheric transport from source areas.[20,109,111,112] Qualitatively, it can be reasonably inferred that a significant fraction of the methylmercury in the aquatic biota of remote or semiremote regions, including marine systems, is derived from anthropogenic mercury entering the aquatic ecosystem or its watershed in atmospheric deposition.[20,94,101,111,113–118] In northern Wisconsin, for example, the total annual atmospheric deposition of mercury to an intensively studied, semiremote seepage lake with no surface inflow and very little groundwater inflow averaged about 0.1 g/ha during 1988 to 1990, an input sufficient to account for the mass of mercury in water, fish, and depositing sediment.[94,114,119]

Concentrations of methylmercury in aquatic biota at remote and semiremote sites have probably increased globally during the past 150 years in response to anthropogenic releases of mercury into the environment. Substantial increases in methylmercury contamination of marine food webs in the North Atlantic Ocean, for example, were revealed by analyses of feathers from two fish-eating

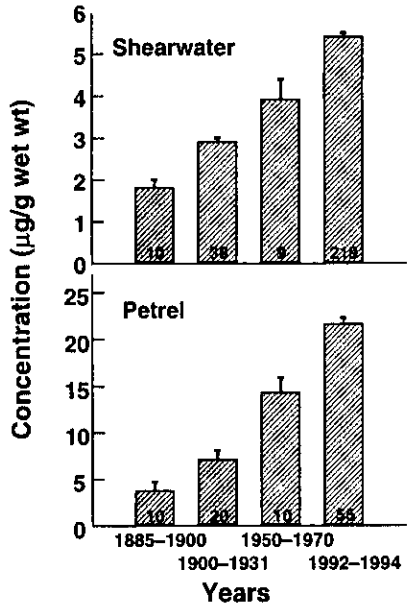

Figure 16.1 Historical trends of increasing methylmercury concentrations (mean ± 1 standard error, with sample size denoted near the bottom of each bar) in feathers of two species of fish-eating seabirds obtained from the North Atlantic Ocean during 1885 to 1994.[118] Monteiro and Furness[118] determined organic mercury in feathers from museum specimens of the birds to avoid potential errors associated with postmortem contamination of the museum samples with inorganic mercury.

seabirds sampled from 1885 through 1994 (Figure 16.1).[118] The long-term increase in concentration of methylmercury averaged 1.9% per year in Cory's shearwater (*Calonectris diomedea borealis*) and 4.8% per year in Bulwer's petrel (*Bulweria bulwerii*).[118] Monteiro and Furness[118] attributed these increases to global trends in mercury contamination, rather than local or regional sources. Mercury concentrations have also increased during the past century in other species of seabirds.[116]

Quantitatively assessing the relative contributions of anthropogenic and natural emissions to the methylmercury burdens accumulated in biota at remote and semiremote sites is an enormous scientific challenge, partly because of spatial variation in (1) the contribution of natural sources and (2) the biogeochemical transformations and transport of mercury on the landscape.[42] The drainage basins onto which anthropogenic mercury is deposited can vary spatially in many respects. First, there is variation in the natural geologic abundances of mercury in bedrock, soils, sediments, and surface waters.[120–122] Second, surface waters within a region can differ spatially and temporally in the extent to which they receive total mercury and methylmercury exported from the drainage basin.[92,93,101,107,108,123–125] Third, the extent to which inorganic mercury present in aquatic ecosystems is converted to methylmercury can vary considerably, even on spatial scales of a few kilometers to tens of kilometers.[68,92,97,126] To overcome such complexities, new investigations involving the application of stable isotopes of mercury[127,128] are being employed to examine the biogeochemical cycling, bioaccumulation, and food-web transfer of "old" vs. newly deposited mercury in ecosystems.[129]

16.3 GLOBAL-SCALE ENVIRONMENTAL CYCLING AND FATE

Our understanding of the biogeochemical cycling of mercury (sources, pathways, and pools) in the environment has increased markedly during the past 10 to 15 years, whether considered in the context of mass balances,[48,130,131] concentrations in environmental media,[20,132,133] or important

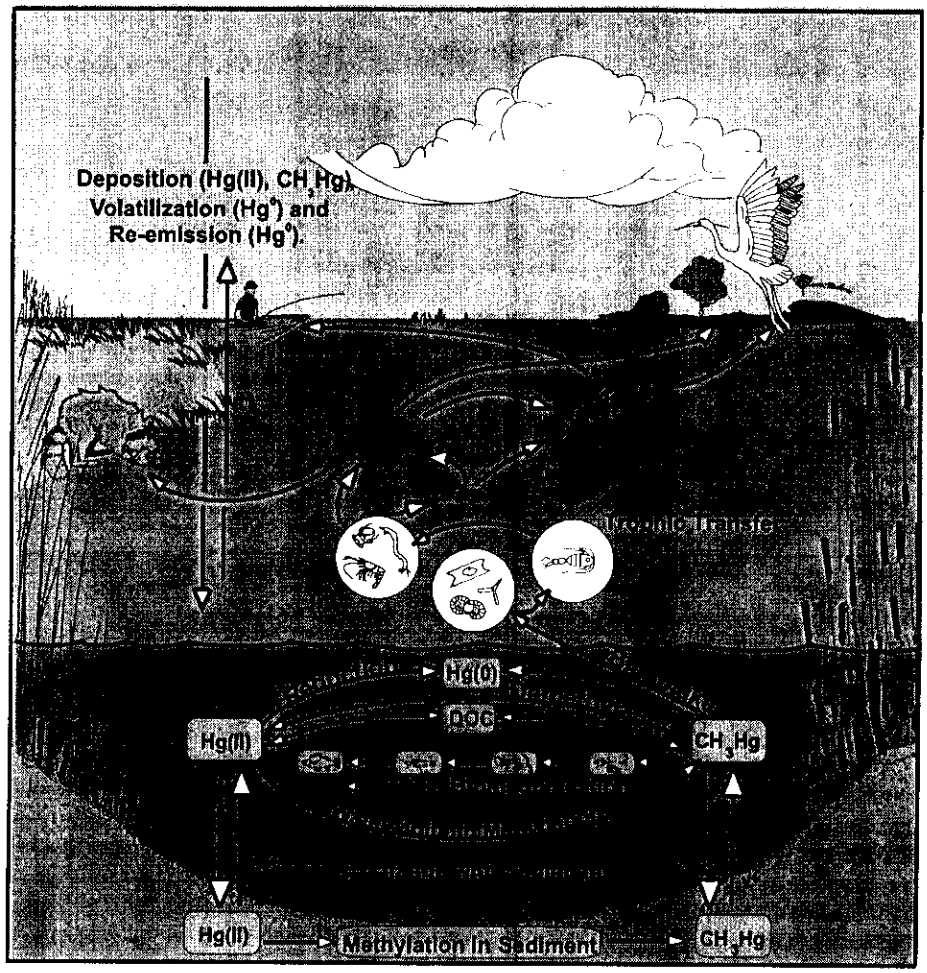

Figure 16.2 A simplified view of the biogeochemical cycling of mercury in an aquatic ecosystem, depicting pathways and processes that influence exposure of biota to methylmercury. In this illustration, mercury enters the ecosystem largely as inorganic Hg(II) in atmospheric deposition. The mercury cycle includes a complex set of biogeochemical processes, of which methylation is most important from an ecotoxicological perspective. Methylmercury is readily bioaccumulated and transferred in food webs and can biomagnify to high concentrations in predatory fish and wildlife. Biotic exposure to methylmercury in the ecosystem is strongly influenced by the net balance between processes that yield methylmercury and make it available to aquatic biota vs. processes that degrade methylmercury or decrease its bioavailability for uptake.

chemical reactions and rates.[134–140] The environmental mercury cycle (Figure 16.2) has four strongly interconnected compartments: atmospheric, terrestrial, aquatic, and biotic. The atmospheric compartment is dominated by gaseous elemental mercury (Hg^0), although Hg(II) dominates the fluxes to the aquatic and terrestrial compartments. The terrestrial compartment is dominated by Hg(II) sorbed to organic matter in soils. The aquatic compartment is dominated by Hg(II)-ligand pairs in water and Hg(II) in sediments, and the biotic compartment is dominated by methylmercury. Mercury is quite reactive in the environment and cycles readily among compartments.

At the global scale, atmospheric processes and pathways dominate the transport of mercury from sources to receptors. The global mercury cycle can be envisioned as a two-way exchange process, in which sources emit elemental mercury (Hg^0) in the gas phase and various species of Hg(II) to

the atmosphere and the atmosphere loses mercury via oxidation of Hg^0 to Hg(II) and the rapid removal of gaseous and particulate species of Hg(II) by wet and dry deposition.[48,139–142] This simple conceptual model reflects the following understanding of atmospheric pathways and processes: (1) that many important sources affecting global mercury cycles (including oceans, fossil fuel combustion, and municipal and medical waste incinerators) emit mostly gaseous Hg^0 and, to a lesser extent, gaseous and particulate species of Hg(II);[10,143,144] (2) that gaseous and particulate forms of emitted Hg(II) are subjected to local and regional removal in dry and wet deposition,[139–141,145] limiting their long-range transport; (3) that divalent mercury can be readily reduced to Hg^0 by natural processes in both terrestrial and aquatic ecosystems;[135,146] and (4) that Hg^0 can be oxidized in the atmosphere to Hg(II), which is efficiently removed in wet and dry atmospheric deposition.[139–142,145]

Mercury deposited onto the land surface in atmospheric deposition is sequestered in terrestrial soils, largely as species of Hg(II) sorbed to organic matter in the humus layer.[131,147,148] Globally, the inventory of mercury in surface soils far exceeds that in the aquatic and atmospheric compartments. The vast majority (947 Mmol) of the estimated total mass of mercury released into the environment during the past century resides in surface soils, compared to 17 Mmol in the atmosphere and 36 Mmol in the oceans.[48] The residence times of mercury in the atmosphere and the oceans are considerably shorter (a year to a few years) than the residence time of mercury in soils. Yet soils should be considered a potential long-term source — as well as a sink — for mercury in the environment, given that the Hg(II) in soils can be reduced and emitted to the atmosphere as Hg^0 or slowly exported to aquatic systems located down gradient.[110,147]

Recent advances in our understanding of mercury in the environment have highlighted the dominant influence of human activities — particularly since the industrial revolution — on the redistribution of global mercury pools, the size of actively cycling pools, and the importance of atmospheric pathways to a global pollution problem.[20,48,106] Anthropogenic emissions have greatly increased the mass of mercury now cycling at the earth's surface and in the atmosphere,[48,106,107,109] causing widespread contamination of terrestrial soils and aquatic sediments.[48,102,133,148,149] Mason et al.[48] estimate that two thirds of the mercury in modern global fluxes is from anthropogenic sources, and the remaining one third is from natural emissions. Soil and sediment are considered to be the dominant sinks for atmospherically derived mercury; however, detailed studies have shown that these enriched pools are susceptible to remobilization via volatilization, leaching, or erosion.[21,50,69,100,110,141] Investigators often find that the more closely they look, the more reactive the existing mercury pools appear to be; for example, St. Louis et al.[150] found that soil-canopy-atmosphere transfer rates were up to three times greater than prior estimates, Friedli et al.[151] showed that forest fires can release mercury from burned areas, and Lalonde et al.[152] showed that recently deposited snow can rapidly lose mercury via reevasion to the atmosphere.

The importance of apportioning between natural and anthropogenic mercury emissions is recognized but very difficult to achieve.[20,48,131,141,153] Initially, the estimated ratio of natural to anthropogenic emissions of mercury may have been underestimated.[141] In Europe, for example, estimated natural emissions of mercury during 1995 were in the range of 250 to 300 tons, only slightly less than the estimate of 342 tons for anthropogenic emissions.[144] In addition, emissions of Hg^0 from areas that are geologically enriched with mercury or affected by mining activities can be substantial.[42,153] The estimation of natural emissions from diffuse sources is greatly complicated by the fact that original sources — whether natural or anthropogenic — cannot presently be distinguished after mercury has been released into the atmosphere and has entered the global biogeochemical cycle.[21,141]

16.4 MERCURY SPECIATION AND ENVIRONMENTAL CONCENTRATIONS

Scientific understanding of mercury speciation in the environment, although far from complete, has increased considerably because of steadily improving analytical and field methods during the

past two decades. Mercury exists in the environment in three oxidation states — Hg(0), Hg(I), and Hg(II) — and for each valence many chemical forms can occur in the solid, aqueous, and gaseous phases. The environmental chemistry of mercury is very complex, and subtle changes in chemical, physical, biological, and hydrologic conditions can cause substantial shifts in its physical form and valence state over time scales ranging from hourly to seasonal.[136,138,152] Here we briefly summarize selective aspects of mercury speciation in the atmospheric, aquatic, and terrestrial environments, focusing on aspects most pertinent to the ecotoxicology of mercury, such as the formation and abundance of methylmercury.

16.4.1 Atmosphere

In most locations, mercury in the atmosphere is mostly (> 95%) gaseous elemental Hg^0, with the remainder composed largely of particulate ionic Hg(II), gaseous divalent mercury (commonly termed "reactive gaseous mercury"), and, on occasion, trace amounts of methylmercury.[141,145] Particulate and reactive gaseous mercury have relatively short travel distances (up to tens of kilometers) and residence times in the atmosphere, whereas gaseous elemental mercury has global-scale transport and an average atmospheric residence time of about 1 year.[48] Recent analyses of air in northern Europe showed that total gaseous mercury averaged 1.98 ng/m^3, whereas particulate mercury and reactive gaseous mercury averaged 56 and 22 pg/m^3, respectively.[154,155] Monovalent mercury is stable only as the dimer (Hg_2^{2+}), which rapidly disproportionates to Hg^0 and Hg^{2+} and is probably only detectable in atmospheric samples at extremely low levels.[135] Over the open oceans concentrations of gaseous elemental mercury increase from the southern hemisphere (~1 ng/m^3 at 60° south) to the northern hemisphere (~3 ng/m^3 at 60° north),[156,157] reflecting the stronger sources of mercury in the northern hemisphere, which is more industrialized and heavily populated than the southern hemisphere.

Reactive gaseous mercury is generally assumed to be $HgCl_2$, although recent research has shown the existence of $Hg(NO_3)_2{\cdot}H_2O$ in the gas phase.[158] After polar sunrise gaseous Hg^0 in the Arctic and Antarctic atmospheres is rapidly depleted via oxidation to reactive gaseous mercury, which increases rapidly in abundance as Hg^0 is depleted.[139,140,159,160] During April and May 2000, reactive gaseous mercury often comprised more than 60% of the total gaseous mercury measured in air over Barrow, Alaska.[160] Reactive gaseous mercury is rapidly removed from the atmosphere via both wet and dry deposition[140,155,160] and is considered to be available for methylation once deposited.[140]

16.4.2 Aquatic Environments

The methylation of mercury and subsequent exposure of biota to methylmercury are greater in aquatic environments than in terrestrial environments. Many recent investigations of mercury in surface waters have determined methylmercury, gaseous elemental mercury (Hg^0), and total mercury (defined as the sum of all mercury species recovered from a strongly oxidized sample).[161] A fraction termed "reactive mercury," which is generally equivalent to mercury reducible by stannous chloride, has also been measured; however, such fractions are often poorly defined and difficult to relate to other environmental factors or processes. The recent development of methods for separating colloidal and truly dissolved fractions of inorganic mercury and methylmercury should advance understanding of aqueous-solid phase partitioning of mercury species and possibly bioavailable fractions.[162] Dimethylmercury has been observed in the marine environment, but only at extremely small concentrations (averaging 0.016 ng/L in the North Atlantic).[163] Dimethylmercury has not been confirmed in fresh waters, however, and its overall importance in the mercury cycle is unknown. We limit this discussion to the three fractions — total mercury, Hg^0, and methylmercury — that are most commonly reported for water.

Except under rare geochemical conditions, or in the vicinity of strong geologic or anthropogenic mercury sources, the concentrations of all forms of mercury in most natural waters are very low

(picograms to nanograms per liter). Most naturally occurring mercury compounds have very low solubility, although mercury complexes with dissolved organic matter are much more soluble.[164] Among surface waters or within a given lake or stream, the abundances of methylmercury and total mercury can vary widely, and the accurate quantification of their aqueous concentrations requires the steadfast application of trace-metal clean techniques to minimize sample contamination during collection, handling, and analysis, coupled with the application of highly sensitive analytical methods.[132,165] When proper sample collection and preservation protocols are followed, intercomparisons among laboratories that use accepted analytical methods for total mercury and methylmercury yield similar results.[166]

The speciation of mercury in water is most strongly influenced by the aqueous chemical conditions — most notably redox, pH, organic ligands, and inorganic ligands.[165] Inorganic divalent mercury, Hg(II), and methylmercury are strongly influenced by the chemical makeup of the host water and almost entirely form ion pairs with ligands in the aquatic environment.[167] In most oxic, circumneutral surface waters, ion-pair formation for Hg(II) and methylmercury is dominated by dissolved organic matter and chloride.[168,169] Under anoxic conditions, which can occur in sediment porewaters and in the hypoliminia of certain lakes, or anywhere reduced sulfur species are appreciable, inorganic Hg(II) and methylmercury will dominantly be present as sulfide or sulfhydryl complex ion pairs.[50,168,170] The complexation of Hg(II) with sulfide can substantially affect the availability of mercury for methylation by microbes.[171]

Concentrations of total mercury in unfiltered water samples from lakes and streams lacking substantive, on-site anthropogenic or geologic sources are usually in the range of 0.3 to 8 ng/L.[133,172,173] In waters influenced by mercury mining or industrial pollution, concentrations of total mercury are greater, often in the range of 10 to 40 ng/L.[33,133,173–175] Surface waters with high concentrations of humic substances can also have high concentrations of total mercury, demonstrating the importance of natural organic material on solubility and aqueous transport of the metal.[115,123,127,176,177] Surface waters draining areas with high geologic abundances of mercury or with contaminated tailings from mercury or gold mining can exceed 100 or even 1000 ng/L in total mercury.[3,26–28,33,38,41,165,178]

In oxic waters, concentrations of methylmercury are typically within the range of 0.04 to 0.8 ng Hg/L.[33,41,68,93,97,123,124,133,173,179,180] However, concentrations of 1 to 2 ng Hg/L can occur in surface waters affected by either industrial pollution (e.g., chlor-alkali plants)[174,181] or mercury mine drainage.[28,133] The fraction of total mercury present as methylmercury is generally higher in fresh waters than in estuarine or marine systems,[182] which may result from inhibition of methylation by the abundant sulfide in pore waters of brackish water systems[171] or from the generally low level of dissolved organic matter in marine settings.[127] Within a given drainage basin or geographic area the concentrations and yields of methylmercury, as well as the fraction of total mercury present as methylmercury, are typically highest in surface waters that drain wetlands.[68,93,123–125,133,183] The biogeochemical processes contributing to the methylmercury-wetland association are under investigation; however, it is evident that biogeochemical conditions in wetlands are favorable for methylation and that complexation of methylmercury with the abundant natural organic matter in wetlands can facilitate its export to waters downstream. Methylmercury generally accounts for about 0.1 to 5% and seldom exceeds 10% of the total mercury present in oxic surface water.[123,133,179] Under anoxic conditions, however, methylmercury can be one of the dominant species of mercury present, and concentrations can exceed 5 ng Hg/L.[96,100,179,184]

Early measurements of Hg^0 in fresh waters showed concentrations ranging from about 0.01 to 0.10 ng/L, which led to a conclusion of pronounced super saturation of Hg^0 in the water column, usually by a factor of 100 to 500, relative to the overlying air,[185] yielding high estimated rates of Hg^0 volatilization to the atmosphere. More recent investigations involving diel measurements have generally shown strong correlations between, on the one hand, instantaneous Hg^0 in the water column and solar intensity and, on the other, a reequilibration with the atmosphere after sundown, with much lower concentrations of Hg^0 in water at night (about 0.005 ng/L).[138,186] Moreover, the

rapid reoxidation of Hg^0 in surface water has also been demonstrated[187] and, when taken into account, greatly decreases estimated volatilization rates of Hg^0 from surface waters.[188] In marine ecosystems, the evasion of Hg^0 appears to be a geochemically significant efflux of mercury.[48,189]

16.4.3 Terrestrial Environments

Comparatively few data are available on the abundances of total mercury and methylmercury in soils and groundwater in upland settings relative to the substantive information available for surface water, sediment, and peat in aquatic environments. Yet recent estimates indicate that terrestrial soils contain the largest inventories of mercury from natural and anthropogenic emissions.[48,131] In addition, the toxicity, solubility, and volatility of mercury depend highly on its speciation, and such information for soils is scant. Various reductive processes can yield appreciable emissions of Hg^0 from contaminated soils, and the mercury in soils may be cycling more actively than previously thought.[147,153,190,191]

The speciation of mercury in most upland soils is probably dominated by divalent mercury species that are sorbed primarily to organic matter in the humus layer and secondarily to mineral constituents in soil.[131,147] Nater and Grigal,[148] who studied forest soils across the upper Midwest of the United States, found that concentrations of total mercury in humus ranged from about 100 to 250 ng/g dry weight, whereas the mineral horizon just below the humus layer contained about 15 to 30 ng/g. In locations near point sources, especially cinnabar (HgS_s) deposits or abandoned placer mines, mercury concentrations can be considerably higher — generally in the µg/g range.[26,153,192,193] The speciation of mercury in such highly contaminated soils depends on the origin of the mercury itself (most likely Hg^0 used for placer mining and chlor-alkali plants, or HgS_s) as well as the chemistry and texture of the soil. Barnett et al.,[194] for example, observed that liquid Hg^0 released to anaerobic, hydric soils resulted in the formation and long-term stabilization of mercuric sulfide. Cinnabar, on the other hand, seems to be more stable when exposed to the surface as mine tailings or transported down gradient from mining operations, generally maintaining its HgS_s stoichiometry, although surface coatings of secondary mercury compounds have also been observed on weathered cinnabar.[195] Little is known about the relative stability and reactivity of mercury amalgam; however, it probably behaves similarly to elemental mercury in the environment.

Published information on concentrations and speciation of mercury in upland soils is sparse, especially for methylmercury. Forest soils have been rarely analyzed for methylmercury, and reported concentrations are generally low — about 0.2 to 0.5 ng/g in the humus layer and < 0.05 ng/g in the mineral-trophic layer.[150,196,197] Although data are few, the very low concentrations of methylmercury in soils, runoff, and groundwater in upland environments suggest that little methylmercury is produced in upland landscapes.[125,131,198]

16.5 MERCURY METHYLATION IN THE ENVIRONMENT

The methylation of inorganic Hg(II) is the most toxicologically significant transformation in the environmental mercury cycle because it greatly increases the bioavailability and toxicity of mercury and increases the exposure of wildlife and humans to methylmercury. It is not surprising that variation in mercury concentrations in fish of a given size (or age) and trophic level among surface waters lacking direct, on-site sources of mercury can be attributed largely to processes and factors that affect the net production and abundance of methylmercury.[92,96,97,126] Mercury *methylation* is the conversion of inorganic Hg(II) to methylmercury by a methyl-group donor. The conversion of methylmercury to inorganic mercury, regardless of the mechanism, is termed *demethylation*. In general, both of these processes (methylation and demethylation) operate simultaneously in aquatic systems. The detection of methylmercury in sediment samples generally indicates a positive net rate of methylation, i.e., the rate of methylation exceeds that of demethylation; however, the

abundance of methylmercury is not necessarily a good predictor of *in situ* methylation rate, given that influxes of methylmercury from external sources can be significant in some settings. In this section, we review the current understanding of methylating and demethylating agents as well as the locations where these processes operate in the environment.

Mercury can be methylated through biotic and abiotic pathways, although microbial methylation is generally regarded as the dominant pathway in the environment.[134,199,200] More specifically, sulfate-reducing bacteria are considered to be the most important methylating agents in aquatic environments,[97,134,199-202] and the most important sites of methylation by sulfate-reducing bacteria are thought to be oxic-anoxic interfaces in sediments[203,204] and wetlands.[93,97,123,198,205] Methylation also occurs in aerobic marine and freshwaters,[206,207] floating periphyton mats and the roots of some floating aquatic plants,[208,209] the intestines of fish,[210] and the mucosal slime layer of fish;[211] however, these sites are considered to be much less important quantitatively than are anaerobic sediments and wetlands.[212] In sediments, the microbial methylation of mercury is most rapid in the uppermost 5 cm of the sediment profile, where the rate of sulfate reduction is typically greatest; comparatively little methylmercury is produced in deeper sediments.[27,65,97,202,213,214]

To be methylated by sulfate-reducing bacteria Hg(II) must first cross the cell membrane of a methylating bacterium, presumably as a neutral dissolved species.[170,171] Thus, the speciation of inorganic mercury in aqueous and solid phases controls the fraction of the total mercury pool that is available for microbial methylation.[170,171] At certain concentrations, for example, chloride and sulfide seem to increase bioavailability because they bind Hg^{2+} as the neutrally charged species $HgCl_2$ or HgS;[170,215] however, at higher ligand concentrations these ion pairs become charged (e.g., $HgCl_3^-$), and availability for methylation is decreased. Likewise, when Hg^{2+} is bound to large molecules of dissolved organic matter or to particulates (either organic matter or clay), it is considered unavailable for biotic methylation.[65] Within microbial cells methylation can be facilitated through enzymatic and nonenzymatic pathways, which are distinguished by the presence or absence of active microbial metabolism, although both pathways call upon methylcobalamine as the active methyl donor to the Hg^{2+} ion.[216,217] Methylcobalamine is produced by many microbes in the environment and reacts with Hg(II) to form methylmercury outside of cells in anaerobic or aerobic conditions,[218] although Choi et al.[219] found that the process is catalyzed enzymatically and that production rates are much higher within cells.

Comparatively little is known about abiotic methylation, which in simple chemical terms implies the existence in the environment of a methyl donor. Several methyl-donating compounds that are attributed largely to industrial sources can methylate mercury abiotically,[220,221] but anthropogenic methyl donors have been documented for few environmental settings. At high concentrations methylated tin and lead compounds can transfer a methyl group to Hg(II) to produce methylmercury,[222] but these situations are very limited in extent. Most of the literature on abiotic methylation consequently suggests that the most important methyl donors in the environment are humic acids,[223,224] although this topic has been studied little. In most cases, abiotic formation of methylmercury has been strongly linked to temperature, and at ambient conditions the methylation rates reported for most abiotic pathways are small. Falter and Wilken[225] have shown that small amounts of methylmercury can be formed abiotically in sediments at ambient temperatures. Their results have had implications for the analytical procedures being used in many laboratories to determine methylmercury because methylmercury can be formed as an artifact while processing samples with high concentrations (generally in the μg/g range) of inorganic Hg(II).[226,227]

Although two processes — methylation and demethylation — ultimately control the abundance of methylmercury, the process of demethylation has received comparatively little study.[228] Demethylation, or methylmercury degradation, can occur via a number of abiotic and biotic pathways in the environment. Like methylation, demethylation can occur in near-surface sediments via microbial pathways[229] and in the water column via microbial and abiotic pathways.[137,199]

Much of the early literature suggested that the microbial degradation of methylmercury involved a two-step, enzyme-catalyzed process by microbes encoded with the *mer*-operon gene sequence,

also referred to as the *mer* detoxification pathway. The *mer*-operon is widespread in nature and has been found for both Gram-negative and Gram-positive bacteria and under both aerobic and anaerobic conditions,[230] although most investigators have found that this process operates in aerobic conditions.[228] The first step of the *mer* pathway involves cleavage of the carbon-mercury bond by the organomercurial lyase enzyme (encoded by the *mer* B gene) to yield methane and Hg^{2+}. A second step involving the mercury-reductase enzyme (*mer* B encoded) reduces Hg^{2+} to Hg^0, yielding a mercury species that can evade from the system.[228,231]

More recently, an oxidative demethylation pathway has been proposed and confirmed by the presence of CO_2 as the end product of the methyl-group breakdown and does not appear to involve the secondary mercury-reductive step.[229,232] These authors[229,232] have proposed that this pathway is similar to the degradation of methanol or monomethylamine by methanogens. The oxidative pathway has been demonstrated in a wide range of environments including freshwater, estuarine, and alkaline-hypersaline sediments and in both aerobic and anaerobic conditions.[233] In anaerobic sediments of the Everglades, methanogens and sulfate reducers have been identified as the principal anaerobes in the oxidative-demethylation process, and maximal rates are observed in near-surficial sediments, where maximal methylation rates are colocated.[233] The precise mechanisms and triggers that induce the oxidative-demethylation pathway remain unclear because demonstration of the process in pure culture remains elusive. However, examination of both the *mer*-detoxification and the oxidative demethylation pathways across a wide range of mercury-contamination gradients suggest that the *mer*-detoxification pathway predominates in severely contaminated systems, whereas the oxidative pathway is more important in lightly contaminated environments.

The abiotic process of photodegradation of methylmercury in surface waters has been recently examined at a few sites.[125,137,234] It has long been known that methylmercury ion pairs are capable of adsorbing light at appreciable levels and are subject to photolytic breakdown;[235] however, the significance of the photodemethylation process was not recognized until photodemethylation was shown to be quantitatively important in the methylmercury budgets of lakes.[125,234,236] The specific mechanisms causing the degradation of methylmercury in surface waters as well as the factors limiting this process, are unknown, but experimental work suggests that singlet oxygen and peroxide radicals are responsible for the reaction.[237] The mercury end products of photodemethylation have not been determined, and theoretical considerations indicate that any of the three oxidation states are possible.[135,138] Identification of mercury end products is needed to assess the overall effect of photodegradation, given that Hg(II) could be methylated again, whereas Hg^0 could evade from the lake to the atmosphere.

16.6 MERCURY-SENSITIVE ECOSYSTEMS

Some aquatic ecosystems can be classified as mercury-sensitive because seemingly small inputs or inventories of total mercury (e.g., in the range of < 1 to 10 g Hg/ha) can cause significant contamination of fish and wildlife in upper trophic levels with methylmercury. Known mercury-sensitive ecosystems include most wetlands,[93,97,124] low-alkalinity or low-pH lakes,[79,92,94,238] surface waters with upstream or adjoining wetlands,[93,124,239] waters with adjoining or upstream terrestrial areas subjected to flooding,[84,85,96,240] and dark-water lakes and streams.[92,93] One common attribute of mercury-sensitive systems is the efficient conversion of inorganic Hg(II) to methylmercury.[85,92,96,97,133,241] In some cases, concentrations of mercury in game fish inhabiting such ecosystems can equal or exceed concentrations observed in fish from waters heavily contaminated by wastes from industrial point sources such as chlor-alkali plants (Table 16.1).

A recent, but growing, body of evidence indicates that wetlands are mercury-sensitive ecosystems. Wetlands can be important sources of methylmercury on the landscape, given that production and yields of methylmercury in wetland areas can greatly exceed that in other aquatic and terrestrial habitats.[123,124] The production of methylmercury in wetlands can increase greatly during flooding,[96] a periodic event in many wetland systems. A number of ecosystem characteristics probably enhance

Table 16.1 Elevated Mercury Concentrations in Axial Muscle Tissue of Selected Freshwater Game Fishes in North American Waters

Aquatic Environment	Mercury Concentration (μg/g wet weight)	
	Range in Means	Range in Maxima
Waters polluted by chlor-alkali plants[a]	1–5	2–15
Newly flooded reservoirs[a]	0.7–3	2–6
South Florida wetlands[b]	0.4–1.4	2–4
Low-alkalinity lakes[a]	0.5–0.9	1–3

Note: Values shown are based on data reported for northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), largemouth bass (*Micropterus salmoides*), and smallmouth bass (*Micropterus dolomieu*).

[a] From values summarized by Wiener and Spry.[173]

[b] Data for largemouth bass from T. R. Lange (Florida Fish and Wildlife Conservation Commission, Eustis, Florida, USA, personal communication).

the microbial methylation of inorganic Hg(II) in wetlands, including an abundance of labile carbon substrates and dissolved organic matter, anaerobic sediments, high microbial activity, and seasonal water-level fluctuations that can cause oscillating redox cycles.[85,92,96,123,126,133,179,242,243] Yet the quantitative effect of methylmercury production and export from wetland areas on contamination of aquatic food webs in downstream waters supporting fish production, piscivorous wildlife, and recreational fisheries has received little study. Wetlands may differ considerably in their methylmercury-producing potential, and the influence of wetland type on methylmercury yield and the identification of associated controlling mechanisms are areas of needed investigation.

Variation in ecosystem sensitivity to mercury inputs is exemplified in Figure 16.3, which depicts tenfold variation in the mercury content of whole yellow perch (*Perca flavescens*) sampled in 1989 from three nearby lakes in northcentral Wisconsin. The three lakes differed chemically, spanning a spatial gradient in mean pH from about 5 to 7, reflecting the variation in the chemistry of lakes in northcentral Wisconsin, an area with hundreds of lakes with low acid-neutralizing capacity.[244] The three lakes were small seepage basins (no surface inlets or outlets) in rural, mostly forest-covered watersheds having no identifiable on-site anthropogenic or enriched geologic sources of mercury. The low-pH seepage lakes in this area receive very little groundwater inflow; rather, nearly all (> 95%) of their hydrologic inflow is from precipitation falling directly onto the lake surface.[245] Surficial sediments in lakes of the area are enriched with mercury, relative to deeper preindustrial sediments,[246] and the mercury-accumulation rate in sediments deposited about 1990 was three to four times that in the mid-1800s.[107] The three lakes can be regarded as lightly contaminated, with inventories of total mercury in surficial sediments (uppermost 5 cm) ranging from 1.6 to 5.8 g/ha[247] and annual atmospheric inputs of mercury (wet plus dry deposition) averaging about 0.1 g/ha from 1988 to 1990.[114] The yellow perch is one of the more widespread and abundant fishes in area lakes and is an important link in the trophic transfer of mercury in lakes of the region.[248,249] Mercury concentrations in walleyes (a regionally important gamefish) and chicks of common loons (*Gavia immer*, a fish-eating bird) are elevated in area lakes with low pH or low acid-neutralizing capacity,[78,238,250] reflecting variation among lakes in contamination of yellow perch, their preferred prey.[251,252]

16.7 BIOACCUMULATION, BIOMAGNIFICATION, AND BIOLOGICAL EFFECTS

16.7.1 Biomagnification in Food Webs

Aquatic organisms can obtain methylmercury from food, water, and sediment, and they bioaccumulate methylmercury with continued exposure because elimination is very slow relative to the rate of uptake.[253] Methylmercury readily crosses biological membranes and can biomagnify to high

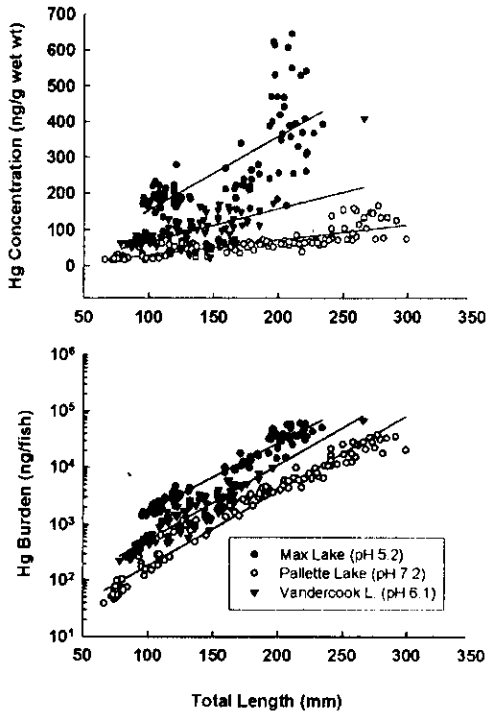

Figure 16.3 Variation in ecosystem sensitivity to mercury inputs, illustrated by differing concentrations (upper panel) and burdens (lower panel, in logarithmic scale) of mercury in whole yellow perch from three nearby seepage lakes in forest-covered watersheds in Vilas County, Wisconsin. Fish in low-pH lakes in this and many other geographic areas have much higher concentrations of mercury than fish in nearby circumneutral-pH lakes that receive similar inputs of mercury in atmospheric deposition. (From: unpublished data for fish sampled in May 1989 by J. G. Wiener, R. G. Rada, and D. E. Powell, University of Wisconsin-La Crosse, River Studies Center, La Crosse, Wisconsin.)

concentrations in aquatic food webs, despite its seemingly low concentrations (< 1 ng Hg/L) in most surface waters.[57,173,254–256] Concentrations of methylmercury in fish, for example, commonly exceed those in ambient surface water by a factor of 10^6 to 10^7.[94,254,256,257]

Most of the mercury in surface waters and sediments is typically inorganic Hg(II), yet the mercury accumulated in fish and higher trophic levels of aquatic food webs is almost entirely methylmercury.[2–4,258–260] Some species of fish-eating seabirds and aquatic mammals exhibit highly variable amounts of inorganic mercury in their internal organs, particularly the liver and kidneys.[258,260–263] The presence of inorganic mercury in piscivorous birds and mammals is generally attributed to the ability of these species to convert methylmercury to the less toxic inorganic mercury via demethylation.[260–263] The presence of inorganic mercury in internal organs and tissues does not indicate dietary uptake or bioaccumulation of inorganic mercury by these organisms.

Biomagnification, defined as the increasing concentration of a contaminant with increasing trophic level in a food web, has been widely documented for methylmercury in aquatic ecosystems.[57,257,264] Patterns of methylmercury biomagnification in aquatic food webs are similar, even among aquatic systems that differ in ecosystem type, mercury source, and pollution intensity. This is illustrated in Table 16.2, which summarizes information from three geographically distant, markedly different aquatic environments: one a coastal marine embayment in Western Australia that was contaminated with mercury from a point source, the second a small seepage lake in northern Wisconsin that received mercury almost entirely via atmospheric deposition, and the third a tropical lake in a remote area of New Guinea. Two patterns characteristic of mercury concentrations in food

Table 16.2 Biomagnification of Methylmercury (MeHg) in Food Webs in Three Substantially Different Aquatic Environments: Princess Royal Harbor, a marine embayment on the south coast of Western Australia that was contaminated with mercury from a super-phosphate plant over a 30-year period;[57,61] Little Rock Lake, a small, temperate seepage lake (no inflowing or outflowing streams) in northern Wisconsin (USA) that received mercury largely from atmospheric deposition directly onto the lake surface;[94] and Lake Murray, a tropical lake in the remote Western Province of Papau New Guinea[257]

Food-Web Component	Australian Marine Embayment[a]		Wisconsin Seepage Lake[b]		Tropical Lake[c]	
	MeHg (ng/g wet wt)	Total Hg present as MeHg (%)	MeHg (ng/g wet wt)	Total Hg present as MeHg (%)	MeHg (ng/g wet wt)	Total Hg present as MeHg (%)
Piscivorous fish	2300	>95	650	>95	392	87
Prey fish	450	93	100	>90	26	55
Invertebrates	150	45	20	29	—	—
Algae	7	10	4	13	<0.3	<1
Water	nd	nd	0.00005	5	0.000067	5

[a] Based on data from Francesconi and Lenanton.[57]

[b] Data for the planktonic food web in the untreated reference basin.[256] The MeHg value for piscivorous fish was the estimated concentration in a hypothetical 5-year-old walleye feeding on yellow perch (the prey fish), based on a regression for nearby lakes.[276]

[c] Data for the planktonic food web, from Bowles et al.[257]

webs are evident in Table 16.2. First, the concentration of methylmercury increases up the food web from water and lower trophic levels to fish. Second, the fraction of mercury present as methylmercury increases with increasing trophic level.

In aquatic invertebrates, methylmercury is much more readily assimilated and bioaccumulated than is inorganic mercury.[265–269] In aquatic organisms in trophic levels below fish, the fraction of total mercury present as methylmercury can vary considerably.[67,87,264,270,271] In 15 northern Wisconsin lakes, for example, percent methylmercury ranged from 9 to 82% in aquatic insects and from 46 to 97% in five taxa of crustacean zooplankton.[264] Mean percent methylmercury varied seasonally in net plankton from 12 lakes in northeastern Minnesota (U.S.), increasing from 20% in spring to 52% in autumn.[272] Percent methylmercury in benthic aquatic insects in two hydroelectric reservoirs in northern Quebec, classified by diet, ranged from 20–25% in detritivores, 30–40% in grazers, 60–85% in grazers–predators, and 95% in predatory dragonflies.[270]

The greatest increase in methylmercury concentration in pelagic food webs, relative to concentrations in water, occurs in the phytoplankton or seston (small living plankton and nonliving particulate matter).[240,257,264,273] Bioaccumulation factors for methylmercury between seston and water, for example, ranged from $10^{4.9}$ to $10^{5.6}$ in tropical Lake Murray, New Guinea,[257] and from $10^{4.8}$ to $10^{6.2}$ in 15 northern Wisconsin lakes.[264] Bioaccumulation factors for methylmercury between herbivorous zooplankton and seston are much smaller, averaging 2.5 ($10^{0.4}$) in 12 northern Wisconsin lakes[266] and about 9 ($10^{0.95}$) in natural lakes and the La Grande 2 reservoir in northern Quebec.[240]

Bioaccumulation factors between concentrations of mercury (largely methylmercury) between piscivorous fish and their prey are also small relative to the orders-of-magnitude increases in methylmercury concentration between seston and water. Ratios of mercury concentrations in piscivorous fish (axial muscle) to those in coexisting prey (whole fish) are typically less than 10, with values ranging from ~4 to 9 in freshwater lakes.[256,274–276] Bioaccumulation factors for mercury reported for seabirds (from analysis of contour feathers) and their prey (from analysis of regurgitated food) are considerably greater, ranging from 125 to 225 and averaging more than 150 in six species of seabirds from the Azores archipelago.[277]

The entry of methylmercury into the base of the food web and its subsequent trophic transfer in the lowest levels are poorly understood. The uptake of inorganic mercury by sulfate-reducing bacteria in the ecosystem is an essential step in the methylation of mercury and a prerequisite to the bioaccumulation and trophic transfer of methylmercury.[255,278] The abundance of methylmercury in the lower trophic levels appears to be strongly linked to the net production or supply of methylmercury (i.e., methylation minus demethylation), with the production of methylmercury being tightly coupled to the rate of sulfate reduction by sulfate-reducing bacteria.[97,202,279]

The dominating influence of methylmercury supply on the contamination of an aquatic food web was illustrated by a flooding experiment in the Experimental Lakes Area of Ontario, Canada.[96,98,100] Experimental flooding of the wetland surrounding Lake 979 was followed by decomposition of inundated vegetation,[280] which rapidly depleted dissolved oxygen and imposed anoxic conditions over the inundated wetland surface, stimulating microbial sulfate reduction and mercury methylation.[96,100] The abundance of methylmercury in surface water, seston, and zooplankton in the lake increased rapidly and markedly (tenfold or greater) in response to increased methylmercury production.[96,98] Concentrations of methylmercury in seston and zooplankton were strongly correlated with those in water, and concentrations in zooplankton were strongly correlated with those in seston as well (all $r^2 \geq 0.85$).[98] Bioaccumulation factors for methylmercury in zooplankton (ratio to concentrations in water) were similar before and after flooding, despite the large changes that occurred in water chemistry, waterborne methylmercury concentration, and zooplankton community structure,[98] indicating that the increased contamination of the planktonic food web resulted directly from the increased supply of methylmercury. This conclusion is further supported by a comparison of the biomagnification of methylmercury in planktonic food webs in the La Grande 2 reservoir to that in natural lakes in northern Quebec.[240] In Lake 979, concentrations of methylmercury also increased after flooding in benthic insects, caged fish (finescale dace, *Phoxinus neogaeus*, which

fed primarily on benthic invertebrates), and nestling tree swallows (*Tachycineta bicolor*, which fed primarily on emergent dipterans).[87,99,281]

Within a given fish population or community, variation in trophic position accounts for much of the variation in methylmercury concentration, both within and among species.[257,282,283] In species with omnivorous feeding habits, such as adult lake trout (*Salvelinus namaycush*), trophic position can vary substantially — even within a single life stage.[284] Thus, concentrations of methylmercury in adult lake trout are strongly correlated with the trophic position in the pelagic food web.[282,283] Moreover, concentrations of methylmercury in organisms at the top of aquatic food webs increase concomitantly with increasing length (number of trophic levels) of the food chain below.

Unlike methylmercury, inorganic mercury is not readily transferred through successive trophic levels and does not biomagnify in aquatic or terrestrial food webs.[254,264,266,285] Consequently, reliance on data from total-mercury determinations for trophic levels below fish (including water, seston, plants, and invertebrates) can produce misleading assessments of food-web contamination and erroneous estimates of potential methylmercury transfer to fish and higher trophic levels.[57,265,286]

16.7.2 Fish

The bioaccumulation of mercury has been more intensively studied in fish than in other aquatic organisms, probably because fish are the primary source of methylmercury in the human diet.[6,12] Nearly all of the mercury in fish muscle and in whole fish is methylmercury.[2,3,57,77,287] There is very little inorganic mercury in either freshwater or marine fish,[2,3,57,77,287] even in aquatic ecosystems with high concentrations of dissolved inorganic mercury.[288] Fish assimilate inorganic mercury much less efficiently than methylmercury from both food and water, and if absorbed, inorganic mercury is eliminated much more rapidly than is methylmercury.[253,289–294]

Dietary uptake probably accounts for more than 90% of the total uptake of methylmercury in wild fishes,[295–298] and fish probably assimilate from 65 to 80% or more of the methylmercury present in the food they eat.[291,294,296] In the laboratory, fish can accumulate high concentrations of methylmercury directly across the gills when exposed to abnormally high concentrations of waterborne methylmercury.[292,299,300] Many of the published laboratory studies on bioaccumulation have exposed test fish to methylmercury concentrations that greatly exceed concentrations of methylmercury in surface waters.[79,173] The mode of uptake (food vs. water) in bioaccumulation experiments, however, seems to have little influence on the distribution of methylmercury among most internal organs and tissues, except that concentrations in the gills are much higher after waterborne (than dietary) exposure and concentrations in the intestines are higher after dietary exposure.[253,300–302]

After crossing the fish gut, methylmercury binds to red blood cells and is transported via the circulatory system to all organs and tissues, readily crossing internal membranes.[294,300,301,303] There is a dynamic internal redistribution of assimilated methylmercury among the tissues and organs of fish exposed to methylmercury in both laboratory and field studies. The masses in the blood, spleen, kidney, liver, and brain decline after exposure to either waterborne or dietary methylmercury ceases, and much of the methylmercury in the body eventually relocates to skeletal muscle, where it accumulates bound to sulfhydryl groups in protein.[290,301–303] Wiener and Spry[173] hypothesized that storage of methylmercury in skeletal muscle serves as a protective mechanism in fishes, given that sequestration in muscle reduces the exposure of the central nervous system to methylmercury.

Within a given fish population, concentrations of methylmercury in muscle tissue or whole fish typically increase with increasing age or body size, a pattern that has been observed repeatedly in surveys of mercury in fishes.[173,253] The increasing concentration with size or age results from the very slow rate of elimination of methylmercury by fish relative to its rapid rate of uptake.[253,293] In a critical analysis of experimental data on methylmercury elimination by fish, Trudel and Rasmussen[293] showed (1) that short-term experiments (< 90 days) substantially overestimate elimination rate, (2) that elimination rate is negatively correlated with body size ($r = -0.65$), (3) that

elimination rate is positively correlated with water temperature (r = 0.77) in long-term (> 90 days) experiments, with a Q_{10} of 1.9, and (4) that the concentration or burden of methylmercury in the fish does not influence the rate of elimination.

The bioaccumulation of methylmercury in fish is influenced by an array of biotic, ecological, and environmental variables. Much of the modern spatial variation in fish mercury levels (within a given trophic level) is attributed to differences among lakes and their watersheds in biogeochemical processes and transformations that control the abundance of methylmercury. The production of methylmercury via the microbial methylation of inorganic Hg(II) in the environment is a key process affecting mercury concentrations in fish.[86,92,97,126,242] It follows logically that factors and processes affecting the microbial production of methylmercury on the landscape will also influence the methylmercury content of fish residing in the ecosystem. Some of the variation in mercury concentrations in fish among northwestern Ontario lakes, for example, is caused by the effect of temperature, or lake size, on the microbial net production of methylmercury in the epilimnia.[126] Mean concentrations in axial muscle of walleye and northern pike (*Esox lucius*) ranged from about 0.7 to 1.1 µg Hg/g wet weight in small (89–706 ha) lakes but were less than 0.4 µg/g in nearby lakes that were larger (2219–34,690 ha) and colder.[126] Specific rates of mercury methylation in the lakes were positively correlated with water temperature, whereas specific rates of methylmercury demethylation (microbial destruction of methylmercury) were negatively correlated with temperature.[304]

The dietary uptake of methylmercury in fish is influenced by their size, diet, and trophic position.[274,283,305–307] In piscivorous species, such as the walleye and lake trout, the methylmercury content of the diet and associated rate of mercury accumulation can increase with age, accelerating abruptly when the fish become large enough to switch from a diet of invertebrates to prey fish.[274,306] In adult fish, females often contain higher mercury concentrations than males because they must consume more food than males to support the energy requirements of egg production.[308,309] The increased feeding rates in females cause greater dietary uptake of methylmercury, and only a small fraction of the accumulated methylmercury is transferred to the egg mass and eliminated during spawning.[287,310,311]

The relative contamination of aquatic food webs with methylmercury can be assessed with information on mercury concentrations in fish of a given species and age,[77,80,81,312–314] particularly if the trophic position of the fish analyzed varies little among the water bodies studied. Within a number of geographic areas of North America, mean concentrations of mercury in same-age yellow perch, for example, vary several fold among midcontinental lakes (and presumably receiving similar rates of mercury deposition). Moreover, mean concentrations of mercury in yellow perch are inversely correlated with lake pH and related chemical variables.[77,81,313,315] Similarly, estimated mercury concentrations in 3-year-old largemouth bass (*Micropterus salmoides*) from 53 Florida lakes varied from 0.04 to 1.53 µg/g wet weight and were correlated with lake pH and related chemical factors.[80]

Methylmercury is neurotoxic and can be very harmful to the central nervous system. In the laboratory, long-term dietary exposure of fishes to methylmercury has caused incoordination, diminished appetite or inability to feed, diminished responsiveness and swimming activity, starvation, and mortality.[173,316,317] Adult fishes were adversely affected in at least two cases of extreme industrial mercury pollution during the past century — Minamata Bay (Japan) and Clay Lake (Ontario). In Minamata Bay, coincident with the poisonings of humans and other organisms, resident fish exhibited symptoms of methylmercury intoxication[318,319] that have been subsequently reported in laboratory experiments.[300,316,317] These symptoms included mortality, severely diminished locomotor activity, impaired escape behavior, emaciated condition, and lesions in the brain.[319] Mercury concentrations in axial muscle of "enfeebled" fishes found floating in seawater in the bay averaged 15 µg/g wet weight and ranged from 8.4 to 24 µg/g in six species.[318]

In grossly polluted Clay Lake in the English-Wabigoon River system (northwestern Ontario), northern pike varying in age from 3 to 8 years had mercury concentrations ranging from 6 to 16 µg/g wet weight in axial muscle tissue.[320] Compared with northern pike from a relatively uncontaminated reference lake, fish from Clay Lake were emaciated, had low hepatic fat stores, exhibited

symptoms of starvation (low levels of total protein, glucose, and alkaline phosphatase in blood serum), and had low serum cortisol levels.[320] After 1 year, contaminated northern pike transplanted from Clay Lake into the reference lake had serum concentrations of total protein, alkaline phosphatase, and cortisol intermediate to those in Clay Lake and reference-lake fish, suggesting partial recovery. Lockhart et al.[320] did not attribute the poor condition of Clay Lake fish directly to effects of methylmercury; however, similar symptoms have subsequently been observed in fish exposed to dietary methylmercury in a laboratory experiment.[317]

Wiener and Spry[173] derived the following critical tissue concentrations in *adult* fish, based on a review of mercury concentrations associated with toxic effects in freshwater fish. In the brain, concentrations of 7 μg/g wet weight or greater probably cause severe, potentially lethal, effects. In mercury-sensitive species, brain-tissue concentrations of 3 μg/g wet weight or greater probably indicate significant toxic effects. For axial muscle tissue, field studies indicate that concentrations of 6 to 20 μg/g wet weight are associated with toxicity. The range for laboratory studies is similar, with sublethal effects or death associated with concentrations in muscle of 5–8 μg/g in walleyes and 10–20 μg/g in salmonid species. Whole-body concentrations associated with sublethal or lethal effects are about 5 μg/g wet weight for brook trout (*Salvelinus fontinalis*) and 10 μg/g for rainbow trout (*Oncorhynchus mykiss*), whereas estimated no-observed-effect concentrations in salmonid species are 3 μg/g for the whole body and 5 μg/g for brain or axial muscle tissue. However, Wiener and Spry[173] cautioned that the toxicity of methylmercury to fish is influenced by factors such as interspecific and intraspecific variation in sensitivity to methylmercury that contribute uncertainty to estimates of critical tissue concentrations. Moreover, the rate of accumulation seems to affect the toxicity of methylmercury in fish.[292] If methylmercury is accumulated slowly, fish can clearly tolerate higher tissue concentrations of mercury, presumably due to the internal transfer and binding of methylmercury to proteins in skeletal muscle (the primary storage site), which decreases exposure of the central nervous system.[173]

Given the high neurotoxicity of methylmercury, the exposure levels causing adverse behavioral effects are probably much lower than exposure levels associated with overt toxicity.[173] Many fish behaviors are sensitive and ecologically relevant indicators of contaminant toxicity, affected at exposure levels much lower than those causing direct mortality.[321–323] The ability of mosquitofish (*Gambusia affinis*) to avoid predation by largemouth bass, for example, was greatly diminished by aqueous exposure to 10, 50, and 100 μg Hg/L (administered as mercuric chloride), concentrations that otherwise did not influence mortality.[321] The neurotoxic effects of exposure to sublethal concentrations of methylmercury can impair the ability of fish to locate, capture, and ingest prey and to avoid predators.[324–326] For example, Fjeld et al.[325] showed that the feeding efficiency and competitive ability of grayling (*Thymallus thymallus*), exposed as eggs to waterborne methylmercuric chloride for 10 days and having yolk-fry with mercury concentrations of 0.27 μg/g wet weight or greater, were impaired when fish were tested 3 years later.

In a critical review, Wiener and Spry[173] concluded that reduced reproductive success was the most plausible effect of mercury on wild fish populations at contemporary exposure levels in aquatic ecosystems with methylmercury-contaminated food webs. They also suggested, based on the limited information available, that the margin of safety between existing and harmful exposure levels may be small for some fish populations. Methylmercury can impair reproduction of fishes by affecting gonadal development or spawning success in the adults or by reducing the hatching success of eggs and the health and survival of embryolarval stages.[64,300,327,328] The embryolarval and early juvenile life stages of fish are typically most sensitive to toxic contaminants,[300] and exposure of embryos to methylmercury can impair competitive ability and foraging efficiency throughout the lifetime of the fish.[325]

Nearly all of the mercury in the developing eggs of fish is methylmercury derived from maternal transfer.[64,287,311] The amount of methylmercury transferred from the female to the developing egg is small relative to the burden of the metal in the adult, yet the methylmercury content of eggs is strongly related to that of the maternal fish.[64,287,311]

Recent experiments showing diminished reproductive success or fitness of fish exposed to environmentally realistic concentrations of methylmercury indicate that some fish populations may be adversely affected by methylmercury.[64,328] Latif et al.[64] examined the effects of both maternally transferred and waterborne methylmercury on embryos and larvae of walleyes from industrially polluted Clay Lake and two atmospherically contaminated lakes in Manitoba. In their study, the hatching success of eggs and the heart rate of embryos decreased with increasing environmentally realistic concentrations of waterborne methylmercury (range, 0.1–7.8 ng/L), whereas methylmercury concentrations in eggs from maternal transfer did not significantly affect egg-fertilization success, egg-hatching success, or the heart rate of embryos. The growth of larval walleyes (measured 8 days after hatching) and the incidence of larval deformities were unrelated to either maternal or waterborne methylmercury in their study.[64]

Hammerschmidt et al.[328] fed fathead minnows (*Pimephales promelas*) diets containing concentrations of methylmercury present in contaminated food webs, maintained the fish through sexual maturity, and examined the effects of dietary and maternally transferred methylmercury on several reproductive variables. In their study, dietary methylmercury affected the overall reproductive performance of adult fathead minnows, whereas maternally transferred methylmercury did not measurably affect the embryos and larvae produced. In the adults, exposure to dietary methylmercury reduced spawning success, delayed spawning, decreased the instantaneous rate of reproduction, and reduced gonadal development (as reflected by the gonadosomatic index) and reproductive effort of females. These responses were caused by dietary concentrations of methylmercury that are equaled or exceeded in the prey of some piscivorous and invertivorous fish inhabiting low-alkalinity lakes and flooded reservoirs.[328] In contrast, the growth and survival of adult fathead minnows in this study were unrelated to dietary methylmercury. Fertilization success, hatching success, 7-day survival, and 7-day weight of larval fathead minnows varied considerably, but these biological endpoints were not correlated with concentrations of mercury in either the diets or carcasses of parental fish.[328]

16.7.3 Birds

The threat of mercury to birds is now largely an aquatic one, given that the probability of exposure of terrestrial birds to high concentrations of organomercurials has diminished substantially since the use of mercury compounds in seed dressings was discontinued. Fimreite[71] reviewed information on bird poisonings caused by mercurial seed dressings. The bioaccumulation and toxic effects of mercury in birds have been reviewed more recently.[329–331] The biomagnification of mercury in aquatic food webs often leads to high concentrations in fish-eating birds.[263,332–334] Moreover, there is evidence that concentrations of methylmercury have increased in some seabirds during the past century and in the past few decades (Figure 16.1).[116,118]

Consumption of fish is the main pathway of methylmercury exposure for birds.[335] Methylmercury, one of the most harmful of contaminants to birds, can adversely affect adult survival, reproductive success, behavior, and cell development.[336] It can also cause teratogenic effects.[337,338] Methylmercury readily crosses the blood-brain barrier[339,340] and is passed from the mother to the eggs.[341–343] When transferred to eggs, nearly 100% of the mercury remains in the methylmercury form and about 85 to 95% is deposited into the albumen.[341]

Incorporation of methylmercury into growing feathers and excretion in the feces are the major routes of mercury elimination in birds,[344] although deposition of mercury into eggs is also an important route for reproducing adult females.[341,343,345] Stickel et al.[346] reported a half-life of mercury in whole bodies of adult male mallards (*Anas platyrhynchos*) of about 12 weeks, and the growth of new feathers toward the end of their 12-week period of study seemed to be responsible for most of the loss. Young birds also excrete methylmercury into their developing feathers.[347]

The concentration of mercury in feathers has been advocated as an indicator of mercury in other avian tissues.[348] Scheuhammer et al.[349] reported significant correlations between mercury in

the feathers and blood of chicks of common loons. Caldwell et al.,[335] who failed to find good correlations between mercury concentrations in chick feathers with those in blood, other tissues, and eggs of double-crested cormorants (*Phalacrocorax auritus*), suggested that more information is needed to establish the dynamics of mercury in feathers vs. other tissues. Unlike fish, in which older, larger individuals tend to have higher concentrations of mercury,[173] birds can annually eliminate much of their body burden of methylmercury through the formation of new feathers.[350]

Fish-eating seabirds seem to be able to demethylate methylmercury, mainly in the liver.[262] The rank order of concentrations of methylmercury in various tissues, averaged over several species of seabirds, was liver > kidney > muscle, and the mean percentages of total mercury present as methylmercury were 35% in liver, 36% in kidney, and 66% in muscle. Furthermore, the percentage of total mercury present as methylmercury decreases as the concentration of total mercury increases.

In double-crested cormorants from Caballo reservoir in New Mexico, where prey fish contained from 0.05 to 0.21 μg Hg/g wet weight, cormorant eggs contained a mean of 0.30 μg Hg/g wet weight, and nestlings that were about 7 to 10 days old had concentrations of 0.36, 0.40, 0.18, and 3.54 μg/g wet weight in blood, liver, muscle, and primary feathers, respectively.[335] Kim et al.,[262] who studied nine species of seabirds, found that both total mercury and methylmercury in nearly all species were higher in liver than in feathers, kidney, and muscle; among these latter three tissues there were no consistent relations. With feathers, it is desirable to know when new feathers were formed because mercury in the diet and body burdens at that time seem to control the deposition of methylmercury into developing feathers.[350] In controlled laboratory studies, where continuous methylmercury diets have been fed, species such as chickens (*Gallus gallus*), ring-necked pheasants (*Phasianus colchicus*), and mallards tend to accumulate the highest concentrations of mercury in liver and kidney, with muscle and brain containing lesser concentrations.[343,351,352]

16.7.3.1 Field Studies on Birds

Observations of high concentrations of mercury in fish-eating birds have prompted field studies to assess effects of methylmercury exposure in wild birds. Mortality and impaired reproduction are two effects observed in controlled laboratory experiments with methylmercury that could decrease exposed populations of wild birds. A number of field studies have shown associations between high mercury levels in the diets or tissues of fish-eating birds and suspected harm. In southern Florida, for example, methylmercury exposure may have contributed to deaths from chronic diseases in great white herons (*Ardea herodias occidentalis*).[353] The livers of herons that died of acute causes, such as collisions with power lines or vehicles, had a mean mercury concentration of 1.77 μg/g wet weight, whereas birds that died showing signs of chronic disease had livers with a mean of 9.76 μg/g mercury. Spalding et al.[353] cautioned that little was known about the history of methylmercury exposure in the dead birds examined in their study. In such cases, the wasting of muscle in sick birds could result in the release of mercury from muscle and its further accumulation in liver.[263]

In the Netherlands, many grey herons (*Ardea cinerea*) died during the winter of 1976.[354] The mercury concentration in the livers of 41 of these dead herons averaged 95.5 μg/g dry weight (~27 μg/g wet weight), with a maximum of 773 μg/g dry weight. Necropsies were performed on 26 of the dead herons, and most were severely emaciated. Van der Molen et al.[354] experimentally exposed herons to methylmercury, and lethality was associated with mercury concentrations in the liver that averaged 500 μg/g and ranged from 415 to 752 μg/g dry weight. Only two of the analyzed dead herons from the field had hepatic mercury levels within this lethal range; however, van der Molen et al.[354] postulated that the observed mortality was caused by the sublethal effects of mercury combined with the stress of cold weather and undernourishment. The authors estimated that mercury levels in livers of 20% of the 26 herons examined were sufficiently high to have caused either lethal or serious sublethal effects. In eastern Canada, Scheuhammer et al.[263] reported that the livers and kidneys of common loons found dead or in a weakened, emaciated condition contained levels of mercury that were high enough to have contributed to their ill health, although the authors noted

that the wasting of muscle and other tissue in the sick and dead loons could have increased the concentrations of mercury in the remaining tissue. The authors also pointed out that, because only low levels of the mercury in the liver were in the methylmercury form, it was questionable whether the loons were affected by methylmercury toxicity.

The embryos of birds and other vertebrate organisms are much more sensitive than the adult to methylmercury exposure.[6,355] The dietary concentrations of methylmercury that significantly impair avian reproduction are only one-fifth of those that produce overt toxicity in the adult,[355] and possible reproductive impairment of wild birds has been reported in a number of field studies.

Newton and Haas[356] examined the levels of several pollutants, including mercury, in eggs of the merlin (*Falco columbarius*). Concentrations of mercury in eggs from wild merlins were related to the number of young raised by the adults, and higher concentrations were associated with fewer young. Nearly all of the mercury in eggs is methylmercury.[341,357,358] The relation between production of young merlins, and mercury exposure was statistically significant but not clear-cut; for example, some of the most contaminated clutches produced 3 or 4 young, whereas some of the nests with low concentrations of mercury in eggs failed completely. Newton and Haas[356] attributed such variable results to variation in individual sensitivity to methylmercury and to the influences of other environmental factors on reproductive success.

Reproductive failure in common loons, a piscivorous aquatic bird, was attributed to dietary mercury exposure linked to contamination of food webs from industrial mercury pollution of the English-Wabigoon River system. Fimreite[332] observed that young common loons were absent along highly contaminated reaches where mercury concentrations were high in adult loons and other aquatic birds. In a comprehensive field study in the same river system, Barr[357] showed a strong negative correlation between the successful use of breeding territories by common loons and mercury concentrations in lakes in a 160-km reach downstream from a chlor-alkali plant. Barr[357] observed that reductions in egg laying and territorial fidelity were associated with mean mercury concentrations of 0.3 to 0.4 μg/g wet weight in prey organisms and with mean concentrations of 2 to 3 μg/g wet weight in loon eggs and the adult brain. Reproductive effects were more severe when concentrations of mercury in prey fish exceeded 0.4 μg/g wet weight.[357]

The North American breeding range of the common loon includes many semiremote and remote lakes in regions where reported mercury concentrations in fish commonly exceed 0.3 to 0.4 μg/g wet weight, the dietary threshold values for reproductive effects estimated by Barr.[357] These regions include the northcentral and northeastern United States and the eastern Canadian provinces of Ontario, Quebec, New Brunswick, and Nova Scotia. Scheuhammer and Blancher,[359] for example, estimated that as many as 30% of the lakes in central Ontario contained prey fish with mercury levels high enough to impair reproduction in common loons, based on the dietary threshold of 0.3 to 0.4 μg/g estimated by Barr.[357]

In northern Wisconsin, another area with many methylmercury-contaminated fish populations, Meyer et al.[82] examined reproductive success of common loons in relation to mercury levels in blood and feathers. Adults and chicks were studied on 45 lakes (mostly seepage lakes) in an area where atmospheric deposition is the dominant mercury source.[360] The mean concentration of mercury in eggs sampled from nests at these lakes was 0.9 μg/g wet weight,[82] an exposure level that has been associated with reproductive impairment in laboratory experiments with mallards.[343] Production of loon chicks was lowest at the lakes where mercury in the blood of chicks was highest. The concentration in chick blood was negatively correlated ($r^2 = 0.56$) with lake pH,[82] a pattern also observed in the methylmercury content of prey fish (Figure 16.3) and game fish in small lakes of the study area.[78,250]

Descriptive field studies typically yield correlational results, which alone are generally insufficient for establishing a causal linkage between toxicant exposure and a biological response, such as reproductive success, because of the potential confounding influence of other, covarying factors.[361–363] For this reason, Meyer et al.[82] did not conclude a cause-and-effect relation between high methylmercury exposure and low production of loon chicks on northern Wisconsin lakes. They

indicated the need to first critically test an alternative hypothesis concerning reproductive success of common loons in northern Wisconsin lakes: that the lower production of loon chicks on low-pH lakes resulted from lesser prey abundance in the low-pH lakes.

Fish assemblages of small seepage lakes used by nesting loons in northern Wisconsin are characterized by low species richness and numerical dominance by a few species, particularly sunfishes (*Lepomis* spp.) and yellow perch.[74,248,249,364,365] The yellow perch, the preferred prey of the common loon,[252] is ubiquitous and typically abundant in these seepage lakes, ranking second in relative numerical abundance (based on catch per unit of effort) only to the bluegill (*Lepomis macrochirus*).[74,248,249,365] Moreover, the yellow perch is an acid-tolerant species, and self-sustaining populations occur in Wisconsin lakes with pH as low as 4.4 standard units.[249,364] Intensive, standardized fish surveys in 12 small lakes in northcentral Wisconsin (fixed sampling effort per lake with four gear types) yielded catches of yellow perch ranging from 285 to 969 fish (mean, 597) in six lakes with low pH (range, 5.1–6.0) and catches ranging from 38–1030 fish (mean, 370) in six lakes with circumneutral pH (range, 6.7–7.5).[74] Two other potential prey-fish species, bluegill and pumpkinseed (*Lepomis gibbosus*), are also abundant in the low-pH lakes.[74] Thus, it is highly improbable that the low production of loon chicks observed by Meyer et al.[82] on low-pH lakes in northern Wisconsin resulted from lesser prey-fish abundance in such lakes. We infer that high methylmercury exposure is a more defensible explanation for the low production of loon chicks on these low-pH lakes.

In Georgia (U.S.), Gariboldi et al.[366] measured mercury levels in prey items regurgitated by nestling wood storks (*Mycteria americana*) at four colonies. The estimated mean concentration in the diet of nestlings at individual colonies ranged from 0.10 to 0.28 μg/g wet weight, equaling or exceeding a lowest observed adverse effect concentration (LOAEC) of 0.1 μg/g wet weight, a value recommended by Eisler[336] as a maximum tolerable dietary concentration for sensitive avian species. The LOAEC of 0.1 μg/g wet weight was derived from laboratory experiments showing that the reproduction and behavior of mallards were affected by a diet containing 0.5 μg Hg/g dry weight (~0.1 μg/g wet weight), administered as methylmercury dicyandiamide. The mean concentration in prey of nestling wood storks was highest (0.28 μg/g wet weight) in an inland colony, where an average of 1.9 young wood storks were fledged per nest — lower than the averages of 2.6 and 2.5 birds fledged per nest at the two coastal colonies, where mean dietary mercury concentrations were 0.10 and 0.19 μg/g wet weight, respectively.[366] Gariboldi et al.[366] stated that it was difficult to separate the effects of dietary mercury exposure from other potential stressors on wood storks such as differences in the abundance of prey among colonies. These authors also noted the uncertainty associated with extrapolating a LOAEC derived from laboratory studies with mallards to wild, fish-eating wood storks. Mercury levels in eggs of wood storks were not measured in this study.

In the English-Wabigoon River system, Fimreite[332] reported a mercury concentration of 3.65 μg/g wet weight in eggs of common terns (*Sterna hirundo*) nesting in Ball Lake, Ontario, where estimated hatching success was less than 27%. In lesser contaminated Wabigoon Lake, mercury averaged 1.0 μg/g in eggs of terns, only seven unhatched eggs were found, and a "large number of fledged young" were observed. The reproductive measurements in this study were made during two visits to each colony and were not systematically collected; however, Fimreite[332] associated the impaired reproduction in terns from Ball Lake with high methylmercury exposure.

The embryos of herring gulls (*Larus argentatus*) seem to be much less sensitive than embryos of the common tern to methylmercury, based on results of Vermeer et al.,[367] who measured mercury in a single egg taken from each of 18 herring gull nests and monitored the subsequent hatching success of the remaining eggs. Mercury levels in whole eggs varied from 2.3 to 15.8 μg/g wet weight. In four eggs, yolk and albumen were analyzed separately, yielding concentrations ranging from 0.9 to 3.5 μg/g in the yolk and from 3.5 to 22.7 μg/g in the albumen. All but two of the remaining eggs in the 18 nests hatched, and those two eggs were in nests where the sampled eggs contained 7.9 and 8.1 μg/g of mercury.[367]

Henny et al.[368] assessed the influence of high concentrations of mercury in eggs on bird reproduction at five national wildlife refuges in the western United States. Concentrations in some

eggs of ducks exceeded 3 μg/g dry weight (~0.6 to 0.8 μg/g wet weight), a concentration near that causing reproductive impairment in laboratory studies with mallards.[343] It was not feasible to relate mercury residues measured in a single egg taken from each duck nest to hatching success of the remaining eggs because of heavy predation on the nests. A small sample of eggs from the nests was, therefore, incubated in the laboratory; eggs with more than 3 μg Hg/g (dry weight) hatched as well as eggs with concentrations less than 3 μg/g.[368]

16.7.3.2 Laboratory Experiments on Birds

An inherent limitation of ecotoxicological field studies stems from the difficulty in isolating the biological effects of methylmercury exposure from the effects of other variables. The presence of other environmental contaminants, for example, can complicate the identification of biological responses to methylmercury. Controlled laboratory experiments are useful for addressing the uncertainties inherent in even the best field studies. A principal objective of many laboratory experiments has been to determine the concentrations of methylmercury in the avian diet or in avian tissues and eggs that are associated with mortality or reproductive failure.

Koeman et al.[369] dosed mice with methylmercury dicyandiamide and fed the mice, which contained about 13 μg Hg/g wet weight, to Eurasian kestrels (*Falco tinnunculus*). Methylmercury poisoning in the kestrels became evident after about 15 days, and mortality began after 21 days. The kestrels suffered demyelinization of the spinal cord, a symptom of methylmercury poisoning. Concentrations of mercury in the kestrels that died or were sacrificed after showing signs of mercury poisoning ranged from 49 to 122 μg/g wet weight in the liver and from 20 to 33 μg/g in the brain.[369] Methylmercury poisoning and mortality also occurred in goshawks (*Accipiter gentilis*) and red-tailed hawks (*Buteo jamaicencis*) fed chicken flesh containing about 4 to 13 μg Hg/g on a wet-weight basis, as methylmercury.[370,371] Livers of the dead goshawks contained from 103 to 144 μg Hg/g wet weight, and brains contained 36 to 51 μg/g.[370] Livers of poisoned red-tailed hawks contained about 19 to 20 μg/g of mercury.[371]

Finley et al.[372] estimated the concentrations of mercury in tissues associated with the death of birds by feeding 40 μg Hg/g, as methylmercuric dicyandiamide, to European starlings (*Sturnus vulgaris*), common grackles (*Quiscalus quiscula*), red-winged blackbirds (*Agelaius phoeniceus*), and brown-headed cowbirds (*Molothrus ater*). After 5 of the 14 birds of each species had died from methylmercury poisoning, 5 survivors were sacrificed, and mercury concentrations in tissues of the dead and surviving birds were compared. The sacrificed birds showed no overt symptoms of methylmercury intoxication. Concentrations in tissues of most dead birds exceeded, but did not differ statistically from, concentrations in the survivors. This study suggested that there is no specific, single concentration of methylmercury in tissues associated with death of the organism. Although no such threshold concentration was evident in their study, Finley et al.[372] considered 20 μg Hg/g wet weight in the tissues as a hazardous concentration.

In another study to determine harmful tissue levels of mercury, Scheuhammer[373] fed diets containing 5 μg Hg/g dry weight, as methylmercuric chloride, to zebra finches (*Poephila guttata*) for 76 days. One fourth of the birds died, and 40% of the survivors exhibited overt neurological signs of methylmercury poisoning including lethargy and difficulty in balancing on their perches. Mercury levels in the brains of finches that died were no higher than levels in birds that showed overt signs of poisoning but did not die. Finches that survived the 76-day exposure period without exhibiting overt symptoms of methylmercury poisoning typically had less than 15 μg Hg/g wet weight in the brain, whereas birds with symptoms had at least 15 μg/g in the brain. Scheuhammer[373] concluded from these and other data that tissue levels of methylmercury associated with neurological effects are similar in birds of different species, size, and dietary mercury level.

In Pekin ducks (*Anas platyrhynchos*) fed 15 μg Hg/g (as methylmercuric chloride), overt signs of mercury poisoning (loss of appetite, decreased mobility, and leg paralysis) appeared in males

after 5 weeks and in females after 8 weeks.[374] Mercury concentrations did not differ between the sexes at death or at the end of the 12-week exposure period; livers of males and females contained 88 and 92 μg/g wet weight, respectively, and brains contained 20 and 23 μg/g.[374] In mallards fed methylmercuric chloride, Pass et al.[375] saw no clear delineation between mercury concentrations in the brains of birds that had developed microscopic lesions in the brain (range, 3.2 to 27.2 μg/g wet weight) and those without detectable lesions (1.8 to 22 μg/g).

Heinz[376] tabulated published concentrations of mercury in the internal tissues of birds that were poisoned, dead, or asymptomatic. He estimated that wet-weight concentrations of mercury associated with harmful methylmercury exposure in adult birds were 15 to 20 μg/g in the brain, 20 to 60 μg/g in the liver, 20 to 60 μg/g in the kidney, and 15 to 30 μg/g in muscle tissue.

The embryos and young of birds are more sensitive to methylmercury than are the adults. Heinz and Locke[340] fed breeding mallards 3 μg Hg/g as methylmercury dicyandiamide, and mercury was accumulated in eggs to mean concentrations between ~5.5 and 7.2 μg/g wet weight in 2 consecutive years. Reproductive success was impaired, and some ducklings died from methylmercury poisoning after hatching. The brains of dead ducklings had mercury concentrations ranging from ~4.9 to 8.7 μg/g wet weight and exhibited demyelination and necrosis characteristic of methylmercury poisoning.[340] Reproduction of black ducks (*Anas rubripes*) fed 3 μg Hg/g as methylmercury dicyandiamide was similarly impaired, with post-hatching mortality of ducklings associated with mercury concentrations between 3.2 and 7.0 μg/g wet weight in the brain.[377]

Concentrations of methylmercury in the maternal diet and in eggs associated with adverse reproductive effects in birds have also been estimated in laboratory studies. Tejning[341] fed chickens a diet containing about 9.2 μg Hg/g dry weight as methylmercury dicyandiamide. Within 3 weeks mercury concentrations in eggs increased to about 25 μg/g wet weight in egg whites and 2 μg/g in egg yolk, and hatching success of exposed eggs decreased to about 10%, relative to about 60% in unexposed controls. When the maternal diet contained about 4.8 μg/g of mercury, administered as methylmercury dicyandiamide, egg whites and yolks contained about 17 and 2 μg/g, and hatching success was 17%.[341] Fimreite[342] fed ring-necked pheasants a diet containing about 3.7 μg Hg/g as methylmercury dicyandiamide. After 12 weeks hatching success was about 10%, compared to about 50 to 55% for controls. In eggs, decreased hatchability was associated with mercury concentrations between 0.5 and 1.5 μg/g wet weight.[342] Borg et al.,[70] who fed breeding pheasants a diet with 15 to 20 μg Hg/g dry weight as methylmercury for 9 days, observed a significant decline in hatching success (55%) relative to controls (74%). Mercury residues in whole eggs associated with this decline ranged from 1.3 to 2.0 μg/g wet weight,[70] in close agreement with the findings of Fimreite.[342]

The diagnosis of methylmercury poisoning in birds based on measured residues of mercury in tissues may be complicated by the influence of other, co-occurring elements that alter the toxicity of methylmercury.[378] In particular, it has been generally believed that selenium protects vertebrate organisms against methylmercury poisoning, even though mercury accumulation in tissues may be increased by selenium.[379]

A notable exception to the presumed protective action of selenium against methylmercury poisoning in wildlife was observed in mallards fed a combination of selenomethionine and methylmercuric chloride.[338] In this experiment, Heinz and Hoffman[338] showed that the toxic effects on the developing bird embryo were much greater when selenium and methylmercury were added jointly to the maternal diet than when methylmercury was added without selenium. In the same experiment, dietary selenium decreased methylmercury toxicity in the adult mallard.[338] Thus, selenium does not seem to protect against reproductive effects of methylmercury.

Few controlled laboratory experiments have been done on the fish-eating birds that are at greatest risk due to methylmercury exposure in the wild. Reproductive experiments with fish-eating birds exposed to dietary methylmercury are urgently needed, given the uncertainties in extending experimental results for laboratory test species to wild, fish-eating birds. Even with laboratory species (such as the mallard, pheasant, and chicken), there is much uncertainty in the threshold concentra-

tions of methylmercury in the maternal diet and in the eggs that elicit reproductive problems. Obtaining information on the reproductive sensitivity of wild, fish-eating birds to methylmercury exposure is perhaps the most pressing research need concerning the avian ecotoxicology of mercury. The combined effects of exposure to methylmercury and other contaminants (particularly selenium and organochlorines), as well as other environmental stressors encountered by wild birds, also merit critical study.

16.7.4 Mammals

Mercury intoxication in wild mammals was first reported in association with the widespread use of organomercurial fungicides as seed dressings during the 1950s and 1960s, when individuals of various wild avian and mammalian species, particularly granivores and their predators, were killed from dietary exposure to high concentrations of mercury.[70] Later, methylmercury intoxication was reported as the cause of death of a wild mink (*Mustela vison*) near a mercury-contaminated river[380] and of a wild otter (*Lutra canadensis*) near a lake contaminated with mercury from a chlor-alkali plant.[381] These cases involved outright mortality of adult mammals, probably in response to high dietary exposure to methylmercury. The sources of mercury that caused these past exposures (i.e., emissions from pulp and paper mills and chlor-alkali plants and usage as seed dressings) have largely been discontinued or greatly reduced. As described earlier in this chapter, however, other regionally and globally significant sources of anthropogenic mercury remain, and the methylation of mercury in the environment and biomagnification of methylmercury to high concentrations in food webs continue. Consequently, piscivorous and other top predatory mammals still risk elevated methylmercury exposure in some aquatic environments.

In this section, we review the effects of dietary methylmercury exposure in wild mammals, the *in vivo* demethylation of methylmercury, and interactions between mercury and selenium in mammals. We discuss evidence linking recent methylmercury exposure to toxic effects in certain wild mammal species and in certain environments of North America. For earlier reviews of mercury toxicology in wild mammals, the reader is referred to Wren,[382] Heinz,[376] Thompson,[329] and Wolf et al.[330]

16.7.4.1 Effects of Methylmercury in Mammals, and Critical Concentrations in Tissues and Diets

Data on tissue concentrations of mercury and methylmercury toxicity are more plentiful for otter and mink than other wild mammals. Combined evidence from wild otter and mink that died after exhibiting signs of methylmercury poisoning[380,381] and from controlled, dietary-dosing experiments on these two species[383–385] indicate that total mercury concentrations in the range of 20 to 100 μg/g wet weight in liver, or > 10 μg/g wet weight in brain, indicate potentially lethal exposure to methylmercury. Reported values for other predatory mammals also fall within these ranges. For example, a fox (*Vulpes vulpes*) that was found staggering and running in circles and that later died had 30 μg/g wet weight of total mercury in its liver and kidneys, and a marten (*Martes martes*) with similar symptoms of methylmercury intoxication had 40 μg Hg/g wet weight in its liver and kidneys.[70] Liver tissue from a Florida panther (*Felis concolor coryi*) suspected of dying of methylmercury poisoning had 110 μg/g wet weight of total mercury.[386] Total mercury concentrations ranging from 37 to 145 μg/g wet weight were found in the livers of feral, domestic cats that died from methylmercury toxicosis.[387] Generally, in the studies cited above only total mercury in tissue was measured, and it was assumed that all or most of the mercury was present as methylmercury, the dominant form to which the animals were exposed. Mammals that die from methylmercury intoxication first exhibit characteristic neurological signs, including some combination of lethargy, weakness, ataxia, paralysis of limbs, tremors, convulsions, and visual impairment.

Chronic exposure to dietary methylmercury concentrations of 1 μg/g wet weight or greater causes neurotoxicity and mortality in adult mink[384,385,388] and otter.[389] Mink die after 3 to 11 months of exposure to 1 μg Hg/g wet weight of methylmercury in the diet.[384–385] Higher dietary concentrations (> 2 μg/g wet weight) hasten the appearance of toxic signs and mortality; however, tissue concentrations of individual mink and otter dying of methylmercury exposure were similar regardless of the methylmercury concentration in the diet, and higher dietary methylmercury concentrations mainly influenced the time required to accumulate toxic tissue concentrations.[388,389] Dietary methylmercury levels of ≤ 0.5 μg Hg/g wet weight are generally not lethal to mink, and consumption of such diets has not caused obvious neurological signs of methylmercury intoxication in mink in controlled feeding experiments.[384,385,390,391]

Comparatively few studies have examined more subtle, sublethal effects of methylmercury in wild mammals. Two studies that examined the effects of dietary methylmercury on reproduction in mink concluded that sublethal exposures (dietary concentrations ≤ 0.5 μg Hg/g) did not adversely affect reproductive variables such as fertility, number of kits born per female, and the survival and growth rates of kits.[385,392] We are unaware of any studies of subtle neurological or neurobehavioral effects of low-level methylmercury exposure in wild mammals such as mink or otter; however, such studies have been done on small mammals used in medical research. Burbacher et al.,[393] who reviewed the medical toxicological literature, concluded that brain-mercury concentrations of 12 to 20 μg/g wet weight during postnatal development are associated with blindness, spasticity, and seizures in small mammals (e.g., rats, mice, and guinea pigs) experimentally exposed to methylmercury; these effects have also been reported in methylmercury-intoxicated mink and otter with similar concentrations in the brain. Lower mercury concentrations (3 to 11 μg/g wet weight) in the brains of small experimental mammals cause behavioral deficits during postnatal development, such as increased activity, poorer maze performance, abnormal auditory startle reflex, impaired escape and avoidance behavior, abnormal visual evoked potentials, and abnormal performance on learning tasks.[393] Similarly, Wobeser et al.[388] concluded that mercury concentrations exceeding 5 μg/g wet weight in the brain, when combined with neurological signs, were consistent with methylmercury toxicity in mink. Mercury concentrations in the brains of free-living otter and mink trapped in Wisconsin and in Manitoba, Ontario, and Quebec were generally in the range of 0.1 to 1.0 μg/g wet weight, although some individuals had 5 to 10 μg/g wet weight.[260,394–397] Results from medical toxicological studies with small mammals indicate that such concentrations in the brain may cause subtle visual, cognitive, or neurobehavioral deficits. Impaired vision and learning ability could be life-threatening to wild, visual predators, given that such dysfunctions could significantly impair ability to catch prey, causing malnutrition, increased susceptibility to disease, or reduced reproductive success.

16.7.4.2 Demethylation and Relationship with Selenium

Information on concentrations of total mercury in certain commonly analyzed tissues, such as the liver, is not sufficient for diagnosing methylmercury toxicity in wild mammals. Methylmercury is the primary and most toxic form of mercury in the diets of piscivores and other top mammalian predators that are associated with aquatic food webs. However, some wild mammals can demethylate methylmercury to varying degrees, and inorganic mercury often accounts for a significant and highly variable fraction of the total mercury present in the liver, kidney, and brain in such species.[260,389,396] Mammals have also been shown to demethylate methylmercury in controlled experiments. In guinea pigs (*Cavia porcellus*), for example, inorganic mercury accounted for 30 and 60% of total mercury in the liver and kidneys, respectively, after 3 weeks of administration of methylmercury.[398]

The inorganic mercury produced by *in vivo* demethylation of methylmercury can gradually accumulate to very high concentrations in association with selenium in certain tissues without

causing any apparent toxicity. Thus, liver-mercury concentrations that would probably be toxic if composed mainly of methylmercury (> 20 µg/g wet weight) may not be toxic if present primarily as Hg-Se complexes. Methylmercury generally predominates when total mercury concentrations in livers of predatory mammals are less than ~9 µg/g wet weight; however, with greater mercury accumulation, an increasingly high proportion of total mercury is often present as inorganic mercury. In marine mammals and aquatic birds that have accumulated high concentrations of mercury in the liver (10 to > 30 µg/g wet weight), more than 85% of total mercury is typically present as inorganic mercury associated with selenium in a molar ratio approximating 1:1.[263,399] Thus, observations of elevated concentrations of total mercury in the liver or kidneys of dead mammals are not sufficient for diagnosing methylmercury intoxication because of demethylation and the subsequent formation of Hg-Se complexes that have relatively low toxicity. Ideally, such diagnoses should be based on information on total mercury, methylmercury, and selenium in the liver, kidneys, and brain. Determination of total mercury in skeletal muscle would also be useful in such assessments because very little demethylation occurs in muscle tissue. In the absence of clinical signs of methylmercury intoxication (e.g., for animals found dead), a mercury concentration exceeding 20 µg/g wet weight in the liver, combined with a concentration exceeding 12 µg/g wet weight in muscle, indicates probable methylmercury intoxication.[383,388,389] Conversely, the same or higher mercury concentration in the liver, in conjunction with low mercury concentrations in muscle, would not be indicative of methylmercury intoxication.

The antagonistic relation between mercury and selenium in biological systems is well known;[379] however, the biochemical mechanisms underlying this antagonism are poorly understood. Studies with rats have demonstrated that selenium protects against or delays the toxicity of methylmercury. Animals that received co-administration of selenium salts with methylmercury had lower mortality, fewer neurological signs of methylmercury intoxication, and better growth rate and weight gain than animals given only methylmercury.[400–404]

In wild mammals, the association between mercury and selenium has been most intensively studied in dolphins. Rawson et al.[405] described pigment granules containing high concentrations of mercury in lysosomes of liver cells of Atlantic bottlenose dolphins (*Tursiops truncates*). All animals with mercury-containing granules had concentrations of total mercury in the liver exceeding 61 µg/g wet weight, whereas animals without pigment had concentrations of less than 50 µg/g. Rawson et al.[405] did not suggest that these mercury-containing pigments were composed of Hg-Se-protein complexes; however, other studies have demonstrated the presence of such compounds, both as Hg-Se-protein complexes and as insoluble HgSe (tiemannite) granules, in liver cells of dolphins.[406–409] As reported for other mammals with high mercury accumulation, dolphins with the highest concentrations of total mercury in the liver (> 100 µg/g wet weight) typically have the lowest percentages of methylmercury (<10 percent of total mercury) and also have high selenium concentrations in the liver.[410] These Hg-Se compounds, which are much less toxic than methylmercury, are very stable and have a long biological half-life, accumulating to high concentrations in older individuals.[405,411] Low concentrations of mercury in the liver of dolphins, and perhaps other marine mammals, are present primarily as methylmercury; however, there is an apparent threshold above which the speciation of mercury is altered by *in vivo* demethylation and concurrent accumulation of selenium with mercury in stable, insoluble complexes.[409] Such Hg-Se complexes have apparently not been reported or studied in otter or mink.

Evans et al.[260] measured concentrations of total mercury and methylmercury in the brain, kidney, liver, and fur of apparently healthy wild otter and mink from Ontario, Canada and reported a greater percent of inorganic mercury in otter than in the same tissues in mink. Mink and otter had comparable levels of total mercury (e.g., in liver, 0.85 to 3.5 µg/g wet weight in mink and 0.87 to 2.3 µg/g in otter), but the mercury in soft tissues of mink was from 80 to 90% methylmercury, whereas the methylmercury fraction ranged from 56 to 81% in otter, leading Evans et al.[260] to suggest that otter are more able than mink to demethylate methylmercury. Wren et al.[396] also reported higher proportions of methylmercury in liver tissue of otter, relative to mink, in animals

collected throughout Ontario. Concentrations of mercury and selenium were not correlated in the livers of Ontario otter.[396]

16.7.4.3 Hazard Assessment Studies

Environmental mercury exposure has occasionally been implicated as a possible contributing factor to population declines in mink and otter. Osowski et al.,[412] who noted that mink were completely absent in parts of the Atlantic coastal plain of Georgia, North Carolina, and South Carolina (southeastern United States), where they were historically abundant and where high-quality habitat remained available, assessed the potential role of 17 environmental contaminants in population declines of mink. Mercury (along with PCBs, DDE, and dieldrin) emerged as a potentially important contaminant influencing mink populations. Concentrations of total mercury in kidneys of mink from areas of concern were as high as 25 μg/g wet weight, compared to < 4 μg/g wet weight in reference areas.[412] Notably, the higher mercury levels reported in this study were toxic to mink in controlled feeding experiments.[388]

Giesy et al.[413] conducted a hazard assessment of mercury and other contaminants in mink above and below hydroelectric dams on three rivers flowing into the North American Great Lakes. Based on an assumed dietary no-observed-adverse-effect concentration of 0.05 μg Hg/g wet weight for mink, the hazard assessment concluded that the calculated hazard indices were not high, although mercury levels in fish upstream from the dams were higher than those downstream. Mercury was deemed to be less important than PCBs as a factor potentially affecting Great Lakes mink populations, and Giesy et al.[413] concluded that concentrations of mercury in fish in the rivers examined were probably too low to cause any population-level effect on mink.

Two studies have attempted to assess the risk of adverse effects in otter and mink from environmental mercury and PCB contamination in the Clinch River and Poplar Creek watersheds in Tennessee.[414,415] Mercury in small fish collected in areas of concern averaged about 0.2 to 0.4 μg/g wet weight.[415] Results of Monte Carlo simulations estimating total daily intakes of mercury, integrated with dose-response curves to estimate risks, led Moore et al.[415] to conclude that dietary methylmercury exposure posed a moderate risk to female mink (a 24% probability of at least 15% mortality) within the areas of concern. Similarly, Sample and Suter[414] concluded that there was an 85% probability that mercury exposure in otter in some of the affected sites exceeded the estimated lowest observed adverse effect level for methylmercury.

16.8 DEGRADATION OF ECOSYSTEM GOODS AND SERVICES

As a "good" produced by aquatic ecosystems, fish are a high-quality food resource — high in protein, low in saturated fat, and a source of beneficial omega-3 polyunsaturated fatty acids and antioxidants such as vitamin E.[416] Mercury contamination has clearly diminished the economic, nutritional, and cultural values of the fishery resources produced by many freshwater and marine ecosystems.[417–420] The growing awareness of the mercury problem has prompted increasing efforts to survey mercury contamination of fish, producing information that has, in turn, prompted issuance of additional advisories concerning the consumption of sport fish. In Canada, mercury contamination accounted for more than 97% (2572) of all fish-consumption advisories listed in 1997.[421] Most of the Canadian advisories pertained to surface waters in Quebec and Ontario, whereas New Brunswick and Nova Scotia had province-wide advisories in effect for mercury. In the United States, methylmercury contamination accounted for 79% of all fish- and wildlife-consumption advisories in 2000.[421] Out of 50 states, 41 had advisories attributed to mercury, and the number of statewide fish-consumption advisories issued for lakes, rivers, or coastal waters increased substantially during 1993–2000 (Figure 16.4). In Sweden, an estimated 40,000 of the country's 83,000 lakes contained 1-kg northern pike with mercury concentrations higher than the national guideline of 0.5 μg/g wet weight, and an

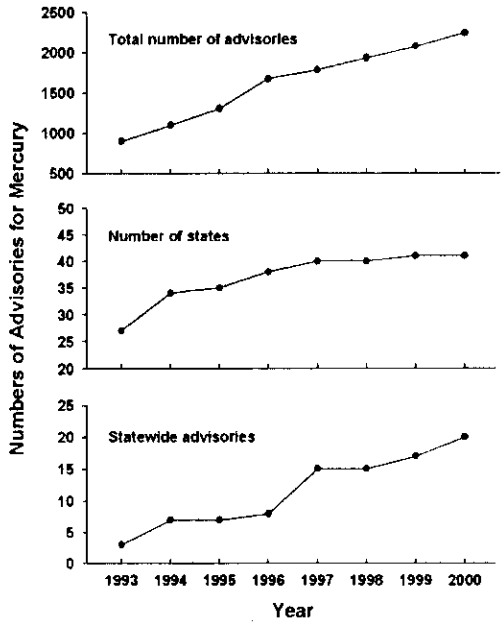

Figure 16.4 Recent trends in the issuance of consumption advisories in the United States caused by mercury in fish and wildlife. Shown are the total number of advisories for mercury (top panel), the number of states issuing advisories (center), and the total number of statewide advisories for surface waters of a given type (bottom), such as coastal waters, lakes, and rivers. Data reflect the status of advisories through the year 2000.[421] In 2001, the states of Pennsylvania and Maryland each issued a statewide advisory (not included above) for mercury in fish.

estimated 10,000 of these lakes contained 1-kg pike with mercury concentrations exceeding the "blacklisting" limit of 1.0 μg/g.[418] In Uppsala County, Sweden, pollution by toxic substances (specifically mercury and cesium-137) ranked as the second greatest anthropogenic threat to lakes.[422]

The economic losses caused by mercury contamination of fishery resources are largely unknown, having been estimated for only a few cases.[417] The adverse impacts of mercury pollution on some indigenous, or aboriginal, peoples who relied on aquatic ecosystems for subsistence via fishing and hunting are multidimensional, encompassing cultural, social, and health effects as well as economic consequences.[419,420,423] For some of these peoples, the consequences of abandoning subsistence fishing and switching to alternative diets, combined with the social and cultural effects of a disrupted lifestyle, have presented a much more severe overall problem than the direct, clinical effects of exposure to methylmercury via consumption of contaminated fish or wildlife.[419,420,423]

The presence of inorganic mercury on the landscape, whether from anthropogenic or natural sources, can devalue some of the services performed by aquatic ecosystems. Much of the inorganic Hg(II) present on vegetated terrestrial areas inundated to create reservoirs for hydropower development, for example, is methylated after flooding and enters the food chain, accumulating to high concentrations in fish in the reservoirs and riverine reaches downstream.[84,85,100,240,242,424–427] In a new reservoir, the inundation of vegetated landscapes is rapidly followed by a rapid transition to anaerobic conditions caused by rapid decomposition of inundated organic matter and the associated depletion of dissolved oxygen near the soil-water interface. After this redox shift, anaerobic microbial communities (including sulfate reducers) proliferate, and the net rate of methylmercury production increases rapidly.[96] In the first 9 years after creation of the La Grande 2 reservoir (part of the La Grande hydroelectric complex in the Canadian province of Québec), concentrations of mercury increased from ~0.6 to 3.0 μg/g wet weight in 70-cm northern pike and from ~0.7 to

2.8 μg/g in 40-cm walleye.[84] In some cases, mercury levels in fish from recently flooded reservoirs have equaled or exceeded levels in fish from surface waters that were heavily contaminated by direct industrial discharges.[242,428] Moreover, the mercury concentrations in the fishery resources of new impoundments may remain substantially elevated for decades after flooding.[84,242,426,429]

The ecosystem services performed by natural and constructed wetlands can also be devalued by mercury. Freshwater wetlands improve water quality by retaining or partially removing many of the constituents from water passing through the wetland system, including suspended solids, bacteria, biological oxygen demand, chemical oxygen demand, certain heavy metals, phosphorus, and nitrogen.[430–433] Constructed wetlands have been widely used for water treatment and are effective at improving water quality in a variety of situations.[432,433] However, wetlands are also quantitatively important on the landscape as sites of methylmercury production, and they can be important sources of methylmercury for downstream surface waters.[68,100,123–125,133,179,183,196,205,434]

The production and export of methylmercury, particularly in wetland systems where the abundance of mercury has been significantly increased by local anthropogenic sources or atmospheric deposition, can impair the water-treatment function and degrade the biological resources of wetlands and downstream waters. In riparian wetlands adjoining the Sudbury River in Massachusetts, for example, contaminated overbank sediments on the floodplain remained an active source of methylmercury for aquatic biota and downstream riverine reaches long after the primary anthropogenic source of mercury was controlled.[60,67–69] In parts of the Florida Everglades, a nationally renowned wetland ecosystem receiving anthropogenic mercury in atmospheric deposition,[435–437] the rates of methylmercury production are very rapid.[97] The fish and wildlife resources in areas of the Everglades having high methylation rates contain high levels of methylmercury,[89,438–441] diminishing the value of the fishery and posing a threat to wildlife in upper trophic levels.[438,442–444]

16.9 MERCURY POLLUTION — A CONTINUING SCIENTIFIC CHALLENGE

Environmental mercury research remains an area of substantive scientific progress and discovery. Virtually hundreds of studies have examined sources, environmental transport, biogeochemical transformations and cycling, bioaccumulation, and biological effects of the metal since global attention first focused on environmental mercury pollution. Amazingly, a variety of recent landmark discoveries indicate that environmental mercury research can be characterized as a relatively "young" field of scientific endeavor.

Examples of recent prominent advances in mercury research include, but certainly are not limited to, the following: (1) the discovery of mercury-sensitive ecosystems characterized by small inventories of total mercury, high rates of mercury methylation, and high concentrations of methylmercury in piscivorous fish and wildlife, (2) the observation that perturbations of the landscape by humans or natural processes (e.g., reservoir creation and prolonged flooding) can markedly increase methylmercury production and contamination of aquatic food webs, (3) the discovery of wetlands as important sites of mercury methylation and export on the landscape, (4) the observation of significant photodegradation of methylmercury in some surface waters, (5) the observation that selenium can worsen — rather than protect against — effects of methylmercury on avian reproduction and developing young, (6) the discovery of highly reactive, gaseous forms of Hg(II) that are rapidly removed from the atmosphere in wet and dry deposition, and (7) observation of the rapid conversion after polar sunrise of gaseous Hg^0 in the polar atmospheres to reactive gaseous Hg(II).

Despite this impressive progress, many significant questions remain concerning the exposure and ecotoxicological effects of mercury in the environment. Our collective appraisal is that progress in assessing the biological and ecotoxicological *effects* of methylmercury exposure is lagging far behind progress in understanding biogeochemical processes and environmental factors that influ-

ence biological *exposure* to methylmercury. In particular, the following topics pertaining to the ecotoxicology of mercury in biota atop aquatic food webs merit intensive scientific study.

- *Critical examination of the reproductive effects of methylmercury on fish, birds, and mammals:* Which, if any, species and populations are being affected by dietary exposure to methylmercury? Is the vertebrate embryo the weak link in reproductive effects in exposed populations, or are other reproductive endpoints (such as territorial fidelity, courtship and other reproductive behaviors, gonadal development, and spawning success) more sensitive?
- *Variation among taxa of piscivorous fish, birds, and mammals in reproductive and early-life sensitivity to methylmercury:* Are fish-eating marine mammals and seabirds protected against the adverse effects of methylmercury exposure, given their apparent abilities to demethylate methylmercury and subsequently store or eliminate the resulting inorganic mercury? Are fish-eating mammals less vulnerable to methylmercury than fish-eating birds?
- *The combined effects of methylmercury and other co-occurring environmental stressors:* How does mercury interact, in an ecotoxicological sense, with other persistent toxic contaminants that bioaccumulate and biomagnify in food webs?

The modern environmental mercury problem, characterized by large geographic scale and enormous complexity, remains a serious challenge to environmental managers and scientists alike. In an ecotoxicological sense, the modern mercury problem can be viewed rather simply as *biotic exposure to methylmercury.* Given this view, the extraordinary challenge facing scientists and environmental managers is to identify approaches that can decrease biotic exposure to methylmercury. Clearly, a sustained, interdisciplinary effort will be needed to address critical questions concerning methylmercury exposure and the associated risks to wildlife and humans dependent on aquatic food webs.

16.10 SUMMARY

Growing awareness of the hazards of mercury exposure prompted widespread reductions in usage and discharges of the metal to many surface waters beginning in the late 1960s. Mercury concentrations in fish and other aquatic biota typically declined in the years and decades following reductions in discharges to industrially polluted waters, although contamination at some sites has decreased slowly and high concentrations persist in fish. Contaminated tailings and alluvium from mining operations are widespread and can remain a source of mercury emissions for decades or centuries. In some basins, contaminated sediment from historic mining sites has been transported to aquatic and floodplain habitats far downstream. Gold mining with the mercury-amalgamation process has resurged in South America, Southeast Asia, China, and Africa in widely dispersed operations that may contribute 10% of modern anthropogenic emissions worldwide.

Since the late 1970s, unexpectedly high concentrations of mercury have been observed in fish from waters lacking on-site anthropogenic or geologic sources of mercury, including low-alkalinity and humic lakes, wetlands, surface waters with adjoining wetlands, waters with adjoining or upstream areas subjected to inundation, and dark-water streams. We classify such systems as mercury-sensitive because seemingly small inputs or inventories of total mercury can cause significant methylmercury contamination in fish and wildlife in upper trophic levels. A common attribute of mercury-sensitive ecosystems is the efficient conversion of inorganic mercury to methylmercury. Concentrations of methylmercury in fish in mercury-sensitive ecosystems can equal or exceed those in fish from industrially contaminated waters.

The modern mercury problem is greatest in aquatic environments, where inorganic Hg(II) can be methylated to methylmercury, the highly toxic form that readily bioaccumulates in exposed organisms and biomagnifies to high concentrations in food webs. Methylation by sulfate-reducing bacteria at oxic-anoxic interfaces in sediments and wetlands is probably the dominant methylation pathway in the environment. Demethylation, or degradation of methylmercury, can occur via a

number of abiotic and biotic pathways in the environment, and photodemethylation can significantly affect methylmercury budgets of lakes.

Emissions from anthropogenic sources and long-range atmospheric transport of mercury have contaminated terrestrial and aquatic environments on a global scale. Analyses of sediment, peat, and glacial ice show that the rate of mercury accumulation at semiremote and remote sites has increased substantially (often by two- to fourfold) since the mid-1800s or early 1900s. It can be reasonably inferred that a significant fraction of the methylmercury in aquatic biota in remote or semiremote waters, including marine systems, is derived from anthropogenic mercury in deposition. In the North Atlantic, for example, concentrations of methylmercury in fish-eating seabirds (and their supporting food webs) increased substantially from 1885 through 1994.

Four compartments — atmospheric, terrestrial, aquatic, and biotic — are interconnected in the global mercury cycle. The atmosphere is dominated by gaseous elemental mercury (Hg^0), but the fluxes between the atmosphere and both aquatic and terrestrial compartments are dominated by Hg(II). The terrestrial compartment is dominated by Hg(II) in soils, the aquatic compartment by Hg(II)-ligand pairs in water and Hg(II) in sediments, and the biotic compartment by methylmercury. Mercury is reactive and moves readily between compartments. Atmospheric processes and pathways dominate global-scale transport from sources to receptors. The global cycle can be envisioned as a two-way exchange, in which sources emit Hg^0 and various species of Hg(II) to the atmosphere and the atmosphere loses mercury via oxidation of Hg^0 to Hg(II) and the rapid removal of gaseous and particulate species of Hg(II) in wet and dry deposition. Mercury deposited onto the land is sequestered in soils, largely as Hg(II) sorbed to organic matter in the humus layer. The global inventory of mercury in soils greatly exceeds that in the aquatic and atmospheric compartments. The Hg(II) in soils can be reduced and emitted to the atmosphere as Hg^0 or slowly transported down gradient; thus, soils are both a sink and a potential long-term source of mercury. Anthropogenic emissions, particularly since the industrial revolution, have greatly increased the size of cycling mercury pools and the importance of atmospheric pathways to a global pollution problem, increasing the abundance of mercury in the atmosphere, soil, sediment, and biota. Natural emissions of Hg^0 can be substantial in areas that are geologically enriched with mercury.

The aqueous abundances of methylmercury and total mercury vary widely. Total mercury in water (unfiltered) ranges 0.3 to 8 ng/L in aquatic systems lacking substantive, on-site anthropogenic or geologic sources, 10 to 40 ng/L in systems influenced by mercury mining or industrial pollution, and can exceed 100 or even 1000 ng/L in systems draining areas with substantive geologic sources or contaminated mine tailings. Methylmercury generally accounts for about 0.1 to 5% of the total mercury in oxic surface water, seldom exceeding 10%. In oxic waters, concentrations of methylmercury are typically in the range of 0.04 to 0.8 ng Hg/L, but can be 1 to 2 ng Hg/L in systems affected by industrial pollution or mercury mine drainage. Methylmercury can be the dominant species under anoxic conditions, and concentrations can exceed 5 ng Hg/L.

Methylmercury readily crosses biological membranes and accumulates in aquatic organisms to concentrations that vastly exceed those in water. Patterns of biomagnification of methylmercury in food webs are similar, even among aquatic systems that differ in ecosystem type, mercury source, and intensity of pollution. The entry of methylmercury into the food web and its concentrations in lower trophic levels are influenced strongly by the supply from methylating environments. Methylmercury concentration increases up the food web from water and lower trophic levels to fish and piscivores. The ratio of methylmercury to total mercury increases with ascending trophic level through fish and can vary greatly in trophic levels below fish. In pelagic food webs, the greatest increase in methylmercury concentration, relative to that in water, occurs between seston and water. Fish accumulate methylmercury mostly via the diet, to concentrations that commonly exceed those in water by 10^6- to 10^7-fold. Inorganic mercury, in contrast to methylmercury, is not readily transferred in food webs and does not biomagnify.

In fish, concentrations of methylmercury increase with increasing trophic position and with increasing age or size, because the rate of elimination is very slow relative to uptake. Much of the

methylmercury in fish is eventually stored in skeletal muscle, bound to sulfhydryl groups in protein; this may serve as a protective mechanism, given that sequestration in muscle reduces exposure of the central nervous system to methylmercury. In the laboratory, long-term dietary exposure of fish to methylmercury causes incoordination, diminished appetite or inability to feed, diminished responsiveness and swimming activity, starvation, and mortality. Fish inhabiting Minamata Bay (Japan) and Clay Lake (Ontario) — both extreme cases of industrial pollution — had very high mercury concentrations and exhibited multiple symptoms of methylmercury intoxication. In laboratory studies, sublethal exposure to waterborne methylmercury can impair the ability of test fish to locate, capture, and ingest prey and to avoid predators. Methylmercury can impair reproduction by affecting gonadal development or spawning success of adult fish, or by reducing the hatching success of eggs and the health and survival of embryolarval stages. Recent experiments have shown diminished foraging efficiency, reproductive success, health, and fitness in fish exposed to environmentally realistic concentrations of methylmercury, indicating that some fish populations may be adversely affected by existing exposure levels.

The sources of mercury responsible for reported deaths of wild birds and mammals (usage in seed grain and emissions from pulp and paper mills and chlor-alkali plants) have been greatly reduced or largely discontinued, yet methylmercury remains a threat to wildlife in upper trophic levels in many aquatic ecosystems. The present pathway of exposure to methylmercury is largely an aquatic one, and reptiles, birds, and mammals atop aquatic food webs often bioaccumulate high concentrations of methylmercury — even at semiremote or remote sites. The principal routes of elimination in birds and mammals are incorporation of methylmercury into growing feathers or hair and excretion in the feces. Some fish-eating birds (particularly seabirds) and mammals can demethylate methylmercury, and inorganic mercury can account for a significant fraction of the total mercury present in the liver, kidney, and brain in such species.

In laboratory experiments with birds and mammals, methylmercury adversely affects survival, reproduction, behavior, and cellular development and causes teratogenic effects. Animals dying from methylmercury intoxication exhibit characteristic neurological signs, including some combination of lethargy, weakness, ataxia, paralysis of limbs, tremors, convulsions, and impaired vision. In adult mink and otter, chronic exposure to dietary methylmercury of 1 μg/g wet weight or greater causes neurotoxicity and mortality. Mortality and impaired reproduction, two endpoints observed in laboratory experiments, could affect populations of birds and mammals exposed to high levels of methylmercury. In field studies, exposure to high levels of methylmercury was a suspected cause of ill health, emaciation, and mortality in studies of wild white herons, grey herons, and common loons. In these cases, the observed mortality may have resulted from the combined effects of sublethal methylmercury exposure and other stressors. In field studies, it is often difficult to isolate the biological effects of methylmercury exposure from those of co-occurring toxic contaminants or other stressors.

In birds and mammals, early life stages are much more sensitive than the adult to methylmercury. Avian reproduction, for example, is significantly impaired at (maternal) dietary concentrations that are only one fifth of those that produce overt toxicity in the adult. In field studies, impaired reproduction has been associated with high mercury exposure in piscivorous birds, including wild merlins, common loons, wood storks, and common terns. In common loons, reductions in egg laying and territorial fidelity have been associated with mercury concentrations averaging 0.3–0.4 μg/g wet weight in prey organisms, and many lakes in the breeding range of this species contain prey-size fish with concentrations equaling or exceeding this estimated threshold value. Reproductive experiments with fish-eating birds exposed to dietary methylmercury are urgently needed, given the uncertainties in extrapolating results from laboratory test species, such as the mallard, to wild piscivorous birds.

Few studies have examined subtle, sublethal effects of methylmercury in wild, piscivorous mammals. Yet medical studies with small mammals have shown that sublethal exposure to methylmercury can cause subtle visual, cognitive, or neurobehavioral deficits that could indirectly affect the survival and reproductive success of visual predators in the wild.

The antagonistic relation between mercury and selenium is well known in toxicology, but the biochemical mechanisms are poorly understood. In dolphins, there is an apparent threshold concentration in the liver above which the speciation of mercury is altered by *in vivo* demethylation followed by storage with selenium in insoluble, stable Hg-Se complexes. Dietary exposure of mallards to selenomethionine and methylmercuric chloride, separately and in combination, showed that selenium decreased methylmercury toxicity in adults. Yet in the same experiment, the adverse effects on the developing mallard embryo were much greater when selenomethionine and methylmercury were added jointly to the maternal diet than when only methylmercury was added.

Mercury contamination is adversely affecting a number of ecosystem goods and services. The most notable of these is the degradation of fishery resources that have substantial economic, nutritional, and cultural value. The economic losses caused by the widespread contamination of fishery resources have apparently not been estimated. For some aboriginal communities in Canada that once relied on subsistence fishing, the impacts of mercury pollution have encompassed adverse cultural, social, and health effects, as well as economic impacts. For some communities, abandonment of subsistence fishing and a change to less healthy diets, combined with the social and cultural effects of disrupted lifestyle, have presented a more severe overall problem than the direct, clinical effects of exposure to methylmercury via consumption of contaminated fish.

Mercury on the landscape can also devalue services performed by aquatic ecosystems. Much of the inorganic Hg(II) on land inundated to create reservoirs for hydropower is methylated after flooding to methylmercury, which rapidly enters the food web and substantively contaminates fish in the reservoirs and reaches downstream for decades. Mercury also devalues the ecosystem services performed by wetlands, which improve the quality of water passing through the wetland system by partial removal of nutrients and certain other constituents. Wetlands are also sites of mercury methylation, and the production and export of methylmercury can impair their water-treatment function and degrade the biological resources in wetlands and downstream waters.

Environmental mercury research is an area of substantive scientific discovery that can be characterized as a relatively young field of scientific endeavor. Our collective appraisal is that progress in assessing the biological and ecotoxicological *effects* of methylmercury exposure is lagging far behind the recent, impressive advances in our understanding of biogeochemical processes and environmental factors that influence biological *exposure* to methylmercury.

In particular, many questions concerning the ecotoxicology of mercury need to be addressed before the population-level effects of methylmercury exposure in fish and wildlife species atop aquatic food webs are fully understood. The modern environmental mercury problem, large in geographic scale and enormous in complexity, remains a daunting challenge for environmental scientists and managers alike.

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EFFECTS OF MERCURY ON NEUROCHEMICAL RECEPTOR-BINDING CHARACTERISTICS IN WILD MINK

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Abstract—Piscivorous wildlife, such as mink (*Mustela vison*), routinely are exposed to mercury (Hg) in their natural environment at levels that may cause adverse behavioral outcomes. The purpose of this study was to determine if a correlation exists between neurochemical receptors and concentrations of Hg in the brains of wild mink. Specifically, receptor-binding assays were conducted to characterize the muscarinic cholinergic (mACh) and dopaminergic-2 (D2) systems in brain tissues collected from mink trapped in the Yukon Territory, Ontario, and Nova Scotia (Canada), and values were correlated with total Hg and methyl Hg (MeHg) concentrations in the brains. A significant correlation was found between Hg (total Hg and MeHg) and mACh receptor density ($r = 0.546$; $r = 0.596$, respectively) or ligand affinity ($r = 0.413$; $r = 0.474$, respectively). A significant negative correlation was found between total Hg and D2 receptor density ($r = -0.340$) or ligand affinity ($r = -0.346$). These correlations suggest that environmentally relevant concentrations of Hg may alter neurochemical function in wild mink, and that neurochemical receptor-binding characteristics can be used as a novel biomarker to assess Hg's effects on wildlife. Given the importance of the muscarinic cholinergic and dopaminergic pathways in animal behavior, further studies are required to explore the physiological and ecological significance of these findings.

Keywords—Wildlife toxicology Neurotoxicology Biomarkers Mercury Mink

INTRODUCTION

Mercury (Hg) is a ubiquitous neurotoxicant and naturally occurring element that exists in multiple allotropic forms [1,2]. Methylmercury (MeHg), the primary organic species of Hg, can readily traverse biological membranes and biomagnify through the aquatic food chain. Consequently, MeHg is a potential risk to high trophic-level piscivorous wildlife, such as mink (*Mustela vison*) [3]. Historical declines of some wild populations of mink are thought to be associated with Hg exposure [4,5]. Additionally, controlled feeding experiments have demonstrated that ranch mink ingesting as little as 1 μg/g dietary MeHg display clinical signs of toxicosis, such as reproductive impairment [6], behavioral changes [7,8], and lethality [7,8]. The effects of Hg on wildlife and ecosystem health is a growing concern because concentrations of Hg measured in the brains of wild mink (range: 0.11–13.4 μg/g wet wt total Hg) [9–11] generally are within one order of concentrations that may cause ill effects (i.e., greater than 5 μg/g wet wt total Hg) [7,12], and global concentrations continue to rise due to industrial activities and long-range atmospheric transport [13–16].

To understand the physiological and ecological risks associated with Hg exposure, feeding trials have been conducted in a variety of avian and mammalian wildlife species [12,17–19]. The endpoints tested in a majority of these studies included bioindicators of health that may be classified as irreversible, such as ataxia and brain lesions. Although these data increase our basic understanding of Hg toxicity, there is a need to develop specific biomarkers to predict the adverse risks associated with chronic exposure to low concentrations of Hg by individuals and populations. Assuming that molecular events at the cellular level precede functional impairments at the organ level, monitoring biochemical changes in the nervous system represents a unique tool to predict possible neurobehavioral outcomes associated with Hg exposure in organisms [20–22].

The ability of wildlife to survive in the environment requires a functional neurological signaling pathway whereby the animal can receive, process, and store information. Two major pathways in neurotransmission are the cholinergic and dopaminergic systems that play critical roles in cognition, somatosensory, and motor function [23,24]. Rodent studies have shown that Hg can disrupt multiple aspects of these pathways including the synthesis, storage, or release of neurotransmitters [25–29], receptor-binding events [30–32], and re-uptake or clearance mechanisms [25,26,28]. Furthermore, studies on fish have shown that exposure of animals to pesticides and heavy metals altered components of the cholinergic [33] and dopaminergic [34] system, which were related to behavioral outcomes. Collectively, these findings suggest that prolonged disruptions in neurotransmission ultimately may alter animal be-

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havior and lend support to the idea that neurochemical changes may be used as biomarkers to study the neurotoxic effects of Hg and other toxicants on wildlife.

Recently, we have demonstrated the versatility and discussed the applications of receptor-binding characteristics to study neurochemistry in epidemiological studies of humans and wildlife [35]. The purpose of the present study is to investigate if there is a correlation between neurochemistry and brain Hg concentrations in wildlife. Specifically, muscarinic cholinergic (mACh) and dopaminergic (D2) receptor-binding characteristics were measured in whole brains of mink collected from different geographical regions of Canada. Receptor data were correlated with concentrations of brain Hg (total and MeHg) to test the following null hypothesis: There is no association between Hg exposure and neurochemical function in wild animals.

METHODS

Chemicals

Radioligands, [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB; 42 Ci/mmol) and [^{3}H]-spiperone (15.7 Ci/mmol), were obtained from NEN/Perkin-Elmer (Boston, MA, USA). Atropine, bovine serum albumin, (+)-butaclamol, ketanserin, and polyethylenimine were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample collection

Animals were collected from licensed trappers during the 2002 to 2003 trapping season from the Yukon Territory (Watson Lake), Southern Ontario (Peterborough and Parry Sound areas), and Nova Scotia, Canada. The gender of each animal was noted and all carcasses were stored at −20°C until processed. The lower jaw was removed to age each animal using cementum annuli readings (Matson's Laboratory, Milltown, MT, USA).

Hg analysis

Concentrations of total Hg and MeHg were quantified as described by Scheuhammer et al. [36]. To quantify total Hg, approximately 0.35 g of freeze-dried brain tissue was digested overnight in concentrated nitric acid, heated for 5 h at 105°C, and diluted eightfold with distilled water. For MeHg analysis, acidic sodium bromide was used to extract the MeHg from a tissue sample into toluene. The Hg complex then was reverse-extracted into the aqueous phase as a thiosulfate conjugate, and this sample was digested and stored in a mixture of strong acids. Concentrations of total Hg and MeHg were measured in the digests using cold vapor atomic absorption spectrophotometry (Hitachi Atomic Absorption Spectrophotometer model Z8200, Tokyo, Japan) at a wavelength of 253.7 nM. Certified reference materials (Dogfish Muscle Certified Reference Material for Trace Metals [DORM-2], Analytical Chemistry Unit, National Research Council, Ottawa, ON, Canada) and sample blanks were included in all analyses for quality control purposes, and all data are expressed as dry weight concentrations, unless otherwise indicated.

Preparation of brain membranes

Brains were excised from each animal and stored at −80°C until membranes were prepared. Frozen tissues were homogenized for 30 s in ice-cold NaK buffer (50 mM NaH_2PO_4, 5 mM KCl, 120 mM NaCl, pH 7.4). The homogenate was centrifuged at 16,500 *g* for 15 min at 4°C, and the resulting pellet was washed twice under the same conditions. The final pellet was resuspended in NaK buffer and aliquots were frozen immediately in liquid nitrogen and stored at −80°C until required. The concentration of protein in the membrane preparation was determined with the Bradford assay [37] using bovine serum albumin as the standard.

mACh receptor-binding assay

One hundred micrograms of membrane preparation were preincubated in NaK buffer for 30 min at 25°C in duplicate. Samples then were mixed with various concentrations (0.01–3.2 nM) of [^{3}H]-QNB, a mACh receptor-specific radioligand, for 60 min at 25°C under constant agitation. The incubation was terminated by rapid vacuum filtration through 1.2-μM glass fiber filters (Millipore, Boston, MA, USA). The filters were washed three times with 3 ml of ice-cold NaK buffer and placed into glass vials. The filters were allowed to dissolve overnight in 5-ml liquid scintillation cocktail (ICN Biomedicals, Aurora, OH, USA). Radioactivity retained by the filters was quantified by a liquid scintillation counter (LKB Wallac 1209 Rackbeta, Turku, Finland) with approximately 68% counting efficiency. Specific binding was defined as the difference in [^{3}H]-QNB bound in the presence and absence of 100 μM atropine, and the total volume in each tube was 1 ml. To reduce nonspecific binding of the radioligand to the filters, filters were soaked for 30 min in 0.1% (weight/volume) polyethylenimine before use.

D2 receptor-binding assay

The receptor-binding assay for the D2 receptor was modified for a 96-well microplate filter system. Twenty micrograms of membrane preparation were preincubated in Tris buffer (50 mM Tris, 5 mM KCl, 2 mM $MgCl_2$, pH 7.4) for 30 min at 25°C in triplicate. Samples then were mixed with various concentrations (0.1–5.6 nM) of [^{3}H]-spiperone, a D2-specific radioligand, for 90 min at 25°C under constant agitation. The incubation was terminated by rapid vacuum filtration through 1.0-μM glass fiber filters (Millipore). Filters were washed three times with 200 μl Tris buffer and placed into glass scintillation vials. The filters were allowed to dissolve overnight in 5-ml liquid scintillation cocktail. Radioactivity retained by the filters was determined as described earlier. Specific binding was defined as the difference in [^{3}H]-spiperone bound in the presence and absence of 100 μM (+)-butaclamol. To reduce nonspecific binding of the radioligand to the filters, filters were soaked for 30 min in 0.5% (weight/volume) polyethylenimine before use, and 50 μM ketanserin (5-HT_2 receptor antagonist) was added to each well to prevent binding of [^{3}H]-spiperone to 5-HT_2 receptors.

Statistical analysis

The critical significance value for all statistical analyses was set at $\alpha = 0.05$. All data are represented as mean ± standard deviation. Data from all receptor-binding studies were curve-fitted using GraphPad Prism (Ver 3.02, GraphPad Software, San Diego, CA, USA) to calculate receptor density (Bmax) and ligand affinity (Kd). To minimize the sum of squares, an *F*-test determined that mACh binding was best fit with a rectangular hyperbolic equation and D2 binding was best fit with a three-parameter logistic equation.

Mercury (total and MeHg) data were log-transformed for statistical analysis (SigmaStat Ver 2.03, SPSS, San Rafael, CA,

Table 1. Mean (±standard deviation) values of Hg levels in mink brains collected across Canada, 2002 to 2003. Values are expressed as a dry weight concentration assuming brain moisture content of 74.6 ± 1.2%

Sampling region	*n*	Total Hg (μg/g dry wt)	MeHg (μg/g dry wt)	MeHg (% of total Hg)
Nova Scotia	27	5.7 ± 5.2[a]	4.9 ± 4.1[a]	90.0 ± 14.9
Ontario	10	1.4 ± 0.6	1.2 ± 0.7	86.8 ± 19.7
Yukon Territory	11	1.2 ± 0.8	1.1 ± 0.8	86.8 ± 14.1

[a] Significant ($p < 0.05$) differences among groups.

USA) because the distribution of Hg data were non-Gaussian as determined by the Kolmogorov-Smirnov test. A Pearson correlation was conducted to explore the relationship between brain Hg (total and MeHg) and neurochemical receptor-binding characteristics (receptor density and ligand affinity). One-way analysis of variance was conducted to examine the relationship between Hg and receptor-binding characteristics among the study regions. Analysis of covariance was conducted to evaluate if any effects were related to age or gender of mink.

RESULTS

Hg analysis

Concentrations of total Hg were measured in two independent laboratories at McGill University (Montreal, QC, Canada) and Trent University (Peterborough, ON, Canada). The mean difference in values was 6.6 ± 4.8% and the cold-vapor atomic absorption spectrophotometry detection limit was 1 μg/L Hg. Mean recovery of Dogfish Muscle Certified Reference Material for Trace Metals (DORM-2) standard reference material was 94.0 ± 4.9% and 93.4 ± 4.3% of the expected value for total Hg and MeHg, respectively. Concentrations of total Hg in the mink brain ranged between 0.27 and 18.84 μg/g and concentrations of MeHg ranged between 0.26 and 13.52 μg/g (Table 1). Concentrations of Hg (total and MeHg) were significantly ($p < 0.001$) higher in Nova Scotia samples compared to those collected from Ontario and the Yukon Territory (Table 1). Methylmercury was measured only at McGill University and accounted for 88.8 ± 15.4% of the total Hg (Table 1). The relationship between total Hg and MeHg was significant ($r = 0.966$, $p < 0.0001$, $n = 44$). Mean moisture content of brain tissue was 74.6 ± 1.2%. No effect of age or gender was found on the concentrations of brain Hg.

Receptor-binding characteristics

Analysis of all mACh receptor-binding data revealed a mean receptor density and ligand affinity of 721.5 ± 227.2 fmol/mg protein and 0.11 ± 0.02 nM, respectively, with highest values recorded in the samples from Nova Scotia (Table 2). Nonspecific binding, as determined by incubation of samples with atropine was less than 5% of total binding at 1 nM [^{3}H]-QNB. Analysis of all D2 receptor–binding data revealed a mean receptor density and ligand affinity of 112.2 ± 32.8 fmol/mg/protein and 1.64 ± 0.33 nM, respectively (Table 2). Although significantly higher D2 receptor density was measured in samples from the Yukon Territory, there were no significant differences in D2 ligand affinity among mink collected from different regions. Nonspecific binding, as determined by incubation of samples with (+)-butaclamol, was 50 to 55% of total binding at 1.8 nM [^{3}H]-spiperone. No effects of age or gender were found on mACh and D2 receptor-binding characteristics.

Correlation of Hg with receptor-binding characteristics

A significant positive correlation was found between total Hg and mACh receptor density ($r = 0.546$, $p < 0.0001$, $n = 47$; Fig. 1A) and ligand affinity ($r = 0.413$, $p < 0.05$, $n = 47$; Fig. 2A). Similar to total Hg, a significant positive correlation was found between brain MeHg and mACh receptor density ($r = 0.596$, $p < 0.0001$, $n = 43$; Fig. 1B) and ligand affinity ($r = 0.474$; $p < 0.001$, $n = 43$; Fig. 2B).

Contrary to the mACh receptor data, a significant negative correlation was found between total Hg and D2 receptor density ($r = -0.340$, $p < 0.05$, $n = 48$; Fig. 3A) and ligand affinity ($r = -0.346$, $p < 0.05$, $n = 48$; Fig. 4A). Correlation of MeHg with D2 receptor density (Fig. 3B) and ligand affinity (Fig. 4B) also was negative, but this association was not statistically significant ($p > 0.05$).

DISCUSSION

Multiple studies have demonstrated that piscivorous wildlife are exposed to Hg via their natural diet, but little is known regarding the physiological and ecological consequences of this long-term exposure. The major finding of this study is that significant differences in mACh and D2 receptor-binding characteristics (receptor density and ligand affinity) can be correlated to concentrations of Hg (total and MeHg) in the brains of wild mink collected across Canada (Figs. 1–4), thus rejecting our null hypothesis. Given the importance of the cholinergic and dopaminergic systems in cognitive processes and motor function, prolonged alterations in receptor properties may precede, and even be used to predict, adverse changes in neurobehavior and animal health.

Concentrations of brain Hg (total and MeHg) measured in this study correspond well with previously published values for mink collected in Ontario [11,38] and Yukon Territory [39]. This is the first report to publish brain Hg concentrations in mink collected from Nova Scotia (Table 1). Significantly higher concentrations of brain Hg (total and MeHg) were measured

Table 2. Mean (±standard deviation) values of neurochemical receptor-binding characteristics (Bmax = receptor density; Kd = ligand affinity) in brain membrane preparations from mink collected across Canada, 2002 to 2003

	Muscarinic cholinergic receptor-binding characteristics		Dopamine-2 receptor-binding characteristics	
Sampling region	Bmax (fmol/mg protein)	Kd (nM)	Bmax (fmol/mg protein)	Kd (nM)
Nova Scotia	1,269.9 ± 378.1A[a]	0.12 ± 0.02A	108.3 ± 30.0	1.59 ± 0.36
Ontario	598.9 ± 203.4	0.11 ± 0.02AB	98.2 ± 40.1	1.69 ± 0.38
Yukon Territory	550.6 ± 88.8	0.09 ± 0.01B	134.5 ± 21.7A	1.74 ± 0.15

[a] A, B = significant ($p < 0.05$) differences among groups.

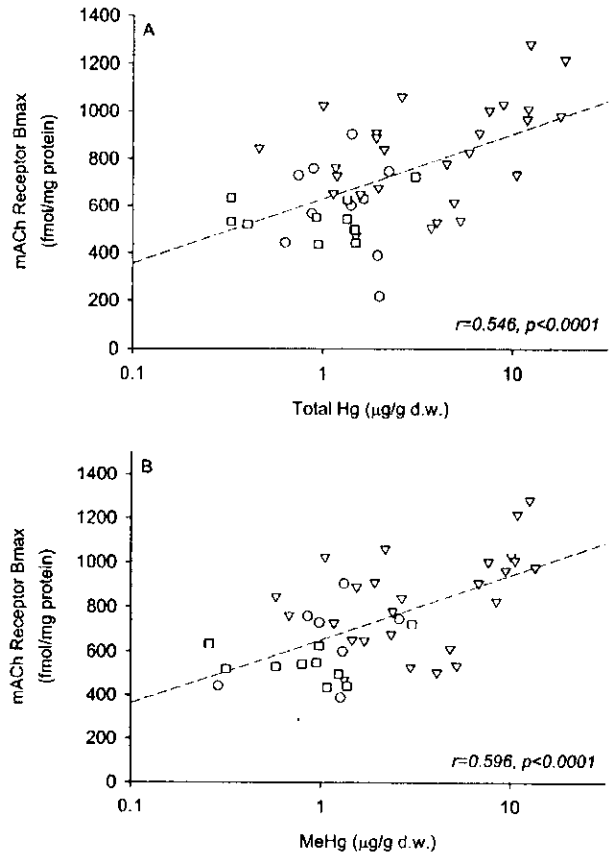

Fig. 1. Relationship between cholinergic muscarinic acetylcholine (mACh) receptor density (Bmax) and levels of total Hg (A) and MeHg (B) in brains of wild mink (*Mustela vison*) collected from Nova Scotia (▽), Ontario (○), and the Yukon Territory (□) in Canada during 2002 to 2003; d.w. = dry weight.

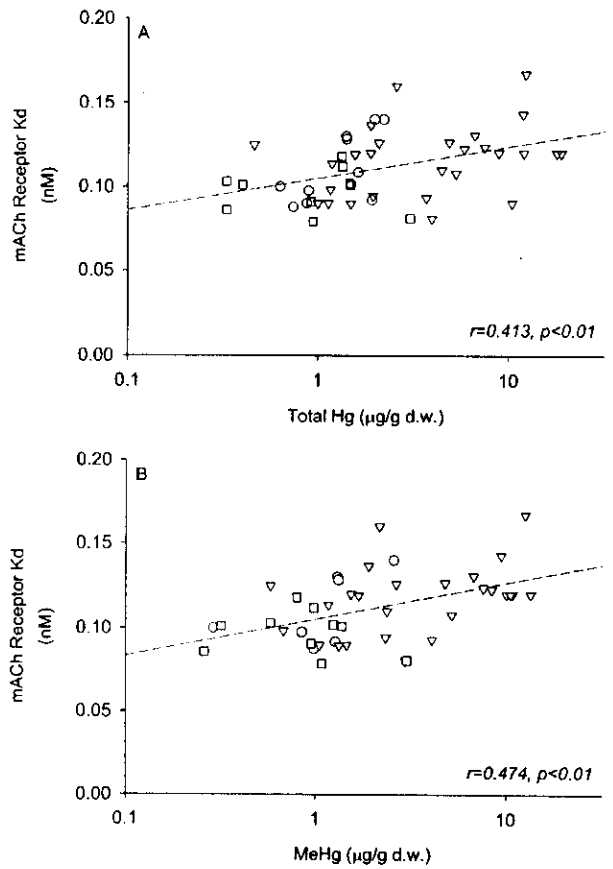

Fig. 2. Relationship between cholinergic muscarinic acetylcholine (mACh) receptor ligand affinity (Kd) and levels of total Hg (A) and MeHg (B) in brains of wild mink (*Mustela vison*) collected from Nova Scotia (▽), Ontario (○), and the Yukon Territory (□) in Canada during 2002 to 2003; d.w. = dry weight.

in mink from Nova Scotia, relative to the Yukon Territory and Ontario, which can be attributed to both the geochemistry of this region [40] and its proximity to industrial point sources that emit Hg [3,41]. Wildlife inhabiting low-alkaline regions, such as Nova Scotia, generally have higher Hg tissue burdens because methylation of inorganic Hg is enhanced under acidic conditions [15]. Mean concentrations of brain MeHg, as a percentage of total Hg, were consistent across the study regions (Table 1) and support previous observations that mink have a limited capacity to de-methylate Hg compared to other piscivorous wildlife [11,42].

Historical reports of Hg poisoning [5,9] and controlled feeding studies [6–8,43] have demonstrated that mink are sensitive to chronic Hg exposure [44]. Given that average concentrations of Hg in North American mink are within one order of magnitude of concentrations measured in severely poisoned mink [3], and MeHg concentrations in many aquatic ecosystems may exceed the U.S. Environmental Protection Agency's (U.S. EPA) derived mammalian wildlife criteria for mink (57 pg MeHg/L) [19], there is a need to explore the subtle effects associated with Hg exposure in the natural environment. After reviewing the available literature, the U.S. EPA [3] recommended a lowest-observable-adverse-effects level of 1.1 μg/g dietary MeHg. This criterion largely was derived from observations that mink fed this ration had brain concentrations of 7.1 to 9.3 μg/g wet weight Hg and resulting neuronal lesions [7]. Others [12] have suggested that brain concentrations of 5 μg/g wet weight MeHg (~19.7 μg/g dry weight MeHg, assuming moisture content of brain to equal 74.6%), may be low enough to cause subtle neurological effects. However, our data demonstrate that significant neurochemical changes exist in wild mink (Figs. 1–4), and levels of Hg (total and MeHg) measured in the brains of these animals were below the concentrations proposed by the U.S. EPA [3] and Wolfe et al. [12] that may cause adverse effects.

Despite several decades of research on the ecotoxological effects of Hg, the only acceptable biomarker for Hg is to quantify exposure by measuring concentrations of Hg in blood, fur, or organs. Although this type of information is necessary for risk assessment, it does not provide much information about the cellular changes that precede functional impairment. A major limitation in wildlife studies is the rapid postmortem degradation of cellular components, such as enzymes and genes, which may be used as possible biomarkers of Hg effect. We have demonstrated recently the versatility of neurochemical receptors in field-based wildlife studies because receptor-binding characteristics were minimally affected by tissue storage temperatures and multiple freeze thaw cycles [35]. These findings, in addition to the results from the current study, lend support to the idea that neurochemical receptor-binding char-

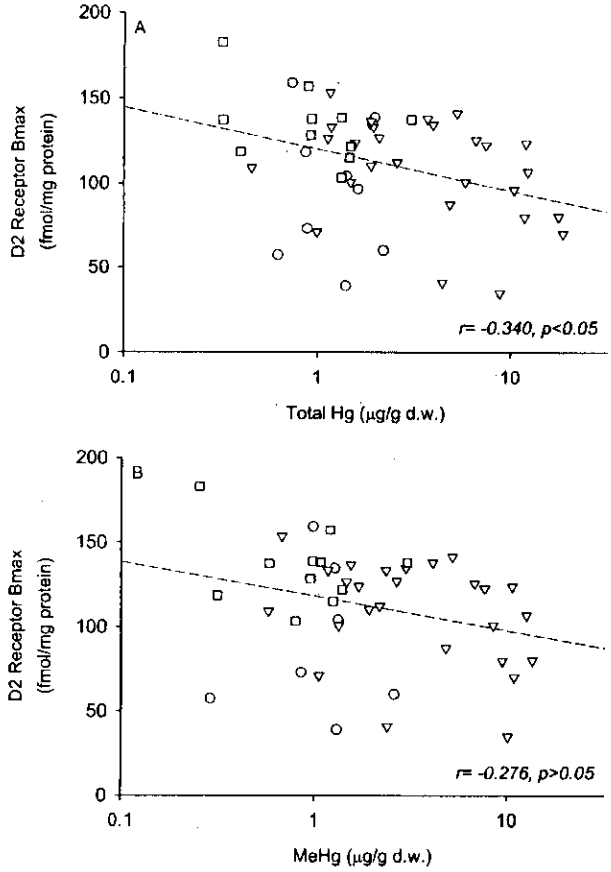

Fig. 3. Relationship between dopamine-2 (D2) receptor density (Bmax) and levels of total Hg (**A**) and MeHg (**B**) in brains of wild mink (*Mustela vison*) collected from Nova Scotia (▽), Ontario (○), and the Yukon Territory (□) in Canada during 2002 to 2003; d.w. = dry weight.

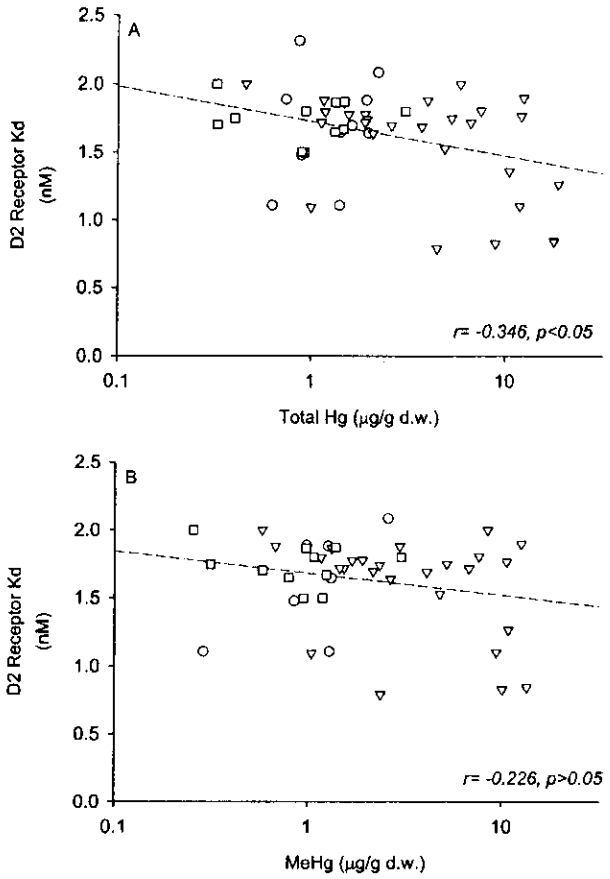

Fig. 4. Relationship between dopamine-2 (D2) receptor ligand affinity (Kd) and levels of total Hg (**A**) and MeHg (**B**) in brains of wild mink (*Mustela vison*) collected from Nova Scotia (▽), Ontario (○), and the Yukon Territory (□) in Canada during 2002 to 2003; d.w. = dry weight.

acteristics are a novel biomarker to assess Hg's neurotoxic effects in wildlife.

It is well-established that Hg can alter neurobehavior in wildlife [12,17,19], but little is known about the mechanisms that mediate these physiological changes. The proper transmission of signals between the animal's external environment and its nervous system is necessary for survival. Mercury is a nonspecific cytotoxic compound [45,46] and rodent studies have shown that organic and inorganic Hg can impair various aspects of neurotransmission. For example, laboratory rats [47] and mice [48] exposed to MeHg had decreased concentrations of brain acetylcholine, the primary agonist of the mACh receptor. Reduced acetylcholine levels, as a result of MeHg exposure, are supported by mechanistic studies demonstrating that MeHg can suppress the activity of choline acetyltransferase [25,26], inhibit the voltage-gated entry of acetylcholine into pre- and postsynaptic nerve endings [49,50], and impair the binding of [^{3}H]-QNB to the mACh receptor [31]. Because Hg may reduce the cellular pool of acetylcholine, up-regulation of the mACh receptor in Hg-exposed mink (Figs. 1 and 2) may represent an adaptive response by these animals to ensure that cholinergic neurotransmission occurs within a normal physiological range. However, the duration an animal can sustain changes in neurochemistry needs to be studied because behavioral changes (e.g., impaired ability to hunt, breed, migrate) may become evident once this latency period is exceeded.

In contrast to the muscarinic cholinergic receptor data, a negative correlation was calculated between Hg and D2 receptor-binding characteristics (Figs. 3 and 4). Mercury has been demonstrated to affect the dopaminergic system in laboratory animals by causing a net increase in cellular levels of dopamine as a result of decreased monoamine oxidase activity [26,51], increased tyrosine hydroxylase activity [25], and spontaneous release of dopamine from neurons [27,29]. In the field, larval mummichogs (*Fundulus heteroclitus*) residing in Hg-contaminated waters had higher levels of dopamine, relative to controls [34]. Knowing that Hg can increase cellular dopamine levels, down-regulation of D2 receptors in high Hg-exposed mink may represent an adaptive mechanism to prevent the hyperstimulation of the dopaminergic system by the animal.

In conclusion, significant Hg-related changes in neurochemistry are measurable in wild mink collected across Canada, and these changes occur at concentrations of brain Hg below values known to cause adverse clinical effects. Because mink are exposed routinely to potentially harmful concentrations of Hg in their natural environment and a functional neurological signaling pathway is essential for animal behavior

and survival, further studies are required to resolve the physiological and ecological significance of these neurochemical changes to individuals and populations. These data also demonstrate that receptor-binding characteristics represent a novel tool to assess the ecotoxicological risks of Hg.

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Effects of Dietary Methylmercury on Reproduction of Fathead Minnows

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We examined effects of dietary methylmercury (MeHg) on reproduction of fathead minnows (*Pimephales promelas*). Juvenile fish were fed one of four diets until sexual maturity (phase 1): a control diet (0.06 μg Hg g^{-1} dry weight) and three diets contaminated with MeHg at 0.88 (low), 4.11 (medium), and 8.46 μg Hg g^{-1} dry weight (high). At sexual maturity, male and female fish were paired, again fed one of the four diets, and allowed to reproduce (phase 2). To assess effects of MeHg during gametogenesis, some fish were fed diets during phase 2 that differed from those during phase 1. Spawning success of pairs fed the same diet during phases 1 and 2 was 75% for controls and 46%, 50%, and 36% for the low-, medium-, and high-MeHg treatments, respectively. Spawning success of pairs fed a contaminated diet during phase 1 and a control diet during phase 2 was 63%, 40%, and 14% for the low-, medium-, and high-MeHg treatments, respectively, whereas exposure to dietary MeHg only during phase 2 did not reduce spawning success. Dietary MeHg delayed spawning, and days to spawning was positively correlated with concentration of total mercury in the carcasses of test fish. MeHg reduced the instantaneous rate of reproduction of fish fed the same diets during phases 1 and 2. Both the gonadosomatic index and reproductive effort of female fish were inversely correlated with mercury in carcasses, whereas developmental and hatching success of embryos, 7-d survival, and 7-d growth of larvae were unrelated to mercury concentrations in parental fish or their diets. MeHg decreased reproduction of adult fathead minnows at dietary concentrations encountered by predatory fishes in aquatic systems with MeHg-contaminated food webs, implying that exposed fish populations could be adversely affected by this widespread contaminant.

Introduction

Little is known about the toxicological effects of methylmercury (MeHg) on the reproduction, growth, and survival of wild fishes (*1–3*). Nearly all of the mercury in adult fish and their eggs is MeHg (*4–7*). MeHg may decrease overall reproductive success of fish by altering gametogenesis and gonadal development of adults or by reducing the hatching success of eggs and the survival of embryolarval stages (*3, 8–11*). Birge et al. (*12*), for example, found that mercury concentrations as low as 0.07–0.10 μg g^{-1} wet weight in eggs of rainbow trout *Oncorhynchus mykiss* were associated with increased mortality; these concentrations are only six to nine times those measured in eggs of rainbow trout from Lake Ontario (*13*) and are within the range measured in eggs of yellow perch *Perca flavescens* from low-alkalinity lakes in northern Wisconsin (*6*).

Few laboratory studies of the effects of MeHg on fish have mimicked exposure conditions in natural waters. Many studies, for example, have used unrealistically high exposures to waterborne MeHg (*2*). More recent investigations have shown that diet is the primary source of MeHg for fish in natural waters (*14, 15*) and that maternal transfer is an important pathway of MeHg exposure for fish embryos (*3, 16*). The amount of mercury transferred from the female to the developing egg is small, yet the mercury content of eggs is strongly related to that of the maternal fish (*6, 7*).

We fed fathead minnows *Pimephales promelas* diets containing concentrations of MeHg present in some aquatic food webs, maintained the fish through sexual maturity, and examined the effects of dietary or maternally transferred MeHg on several reproductive variables. Our principal objective was to examine the effects of MeHg administered via the diet of parental fish on the overall reproductive success of fathead minnows.

Experimental Section

Study Design. The study included four sequential phases corresponding to life stages of the fathead minnow: (phase 1) the juvenile stage until sexual maturity, (phase 2) spawning of mature fish, (phase 3) embryogenesis, and (phase 4) growth of larval progeny. During each phase, we examined effects of either dietary or maternally transferred MeHg on fathead minnows.

Throughout the study, fathead minnows were cultured with methods described by others (*17–19*). Larvae were fed brine shrimp nauplii (*Artemia* sp.) until 45-d post hatch and then fed Starter soft-moist fish food (Nelson and Sons, Inc., Murray, UT). About 3 months after hatching, about 1400 juveniles were randomly placed into each of four 500-L flow-through tanks receiving well water (Figure 1, phase 1).

Phase 1. Juvenile fathead minnows were fed *ad libitum* one of four phase-1 diets, three of which were contaminated with methylmercuric chloride. Mean concentrations (μg g^{-1} dry weight) ± 1 SE of total mercury in the diets were 0.060 ± 0.003 (control), 0.88 ± 0.02 (low), 4.11 ± 0.08 (medium), and 8.46 ± 0.17 (high). Normalized to caloric density, levels of mercury in the test diets spanned those in zooplankton, benthic invertebrates, and small forage fish from low-alkalinity lakes in North America (Table 1). Hence, these dietary concentrations are environmentally realistic and reflect potential dietary exposures of fish in many aquatic systems with MeHg-contaminated food webs.

Phase 2. After fathead minnows became sexually dimorphic (about 240 d post-hatch), mature males and females were paired randomly and assigned to quadrants in one of 15 50-L flow-through breeding aquaria receiving well water (Figure 1). Breeding aquaria were arranged randomly within a large water bath, and each aquarium was partitioned into quadrants with plastic screen. One pair of fish inhabited each quadrant with an acid-cleaned spawning substrate (a half cylinder of PVC pipe). Pairs were fed a phase-2 diet with one of four concentrations of MeHg. To evaluate the effects of dietary MeHg during gametogenesis on reproduction, some fish were fed phase-2 diets that differed from those fed during

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TABLE 1. Mercury Concentrations in Diets Used in This Study in Relation to Those in Zooplankton, Benthic Invertebrates, and Small Prey Fish in Low-Alkalinity Lakes[f]

item	caloric density (Kcal g^{-1} dry wt)	concentration of mercury ($\mu g\ g^{-1}$ dry wt)	concentration of mercury (μg Kcal^{-1})	determined, or probable dominant form, of mercury	ref
control diet	5.16[a]	0.06	0.01	total	this study
zooplankton	5.24	0.06–0.28	0.011–0.053	methyl	(*42*)
zooplankton[b]	5.24	0.007–0.29	0.001–0.056	methyl	(*35*)
benthic invertebrates[b]	4.82	0.001–1.0	<0.001–0.21	methyl	(*43*)
low diet	5.16[a]	0.88	0.17	methyl	this study
whole yellow perch (age-2)	4.3–5.6[c,d]	0.12–1.2[c]	0.02–0.27	methyl	(*44*)
whole smelt (*Osmerus mordax*)	3.1–5.0[c,e]	0.20–2.3[c]	0.04–0.75	methyl	(*45*)
crayfish abdominal muscle	3.3[c]	0.84–2.5[c]	0.26–0.75	methyl	(*46*)
medium diet	5.16[a]	4.11	0.80	methyl	this study
high diet	5.16[a]	8.46	1.64	methyl	this study

[a] Determined by bomb calorimetry. [b] From Lake 632 (reference site). [c] Estimated dry-weight value assuming a whole-body water content of 75%. [d] Based on value reported by Craig (*47*). [e] Based on value reported for fish less than 140 mm standard length (*48*). [f] Unless noted otherwise, caloric densities are based on values reported by Cummins and Wuycheck (*41*).

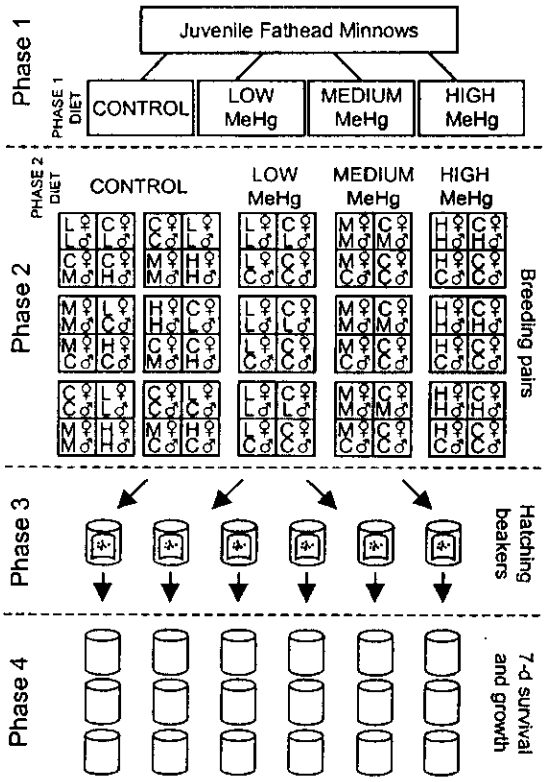

FIGURE 1. Experimental design used to evaluate effects of dietary methylmercury (MeHg) on reproduction of fathead minnows. Letters in quadrants of breeding aquaria (phase 2) represent the MeHg-contaminated diet fed to female (circle over plus sign) and male (circle and arrow) fish during phase 1 of the study (C = control diet with no added MeHg, L = low, M = medium, and H = high).

phase 1. To examine the relative effects of either male or female exposure to dietary MeHg during phase 1, some fish fed a contaminated diet during phase 1 were paired with fish fed the control diet in phase 1. Male and female fish were paired so that every treatment (i.e., phase-1 diet of male × phase-1 diet of female × phase-2 diet of pair) was replicated concurrently and over the 136-d period of phase-2 observation with replacement pairs of fish. Male and female fathead minnows were capable or nearly capable of reproducing when placed into breeding aquaria. The ovaries of female fathead minnows contain eggs in all stages of development, and they spawn repeatedly as the eggs mature (*19*). Male fathead minnows initiate spawning by courting a receptive female to deposit adhesive eggs on the underside of a floating or submerged substrate (*19*, *20*). Oviposited eggs are subsequently fertilized, aerated, and kept clear of detritus by the male fish.

Phase 3. Spawning substrates were examined daily for eggs. Each substrate with eggs was transferred to a 1-L beaker containing aquarium water (Figure 1). Water in the beakers was aerated gently and maintained at 25 ± 1 °C with a photoperiod of 16 h light:8 h dark. To ensure that the gametes were produced or matured while the fish were fed the phase-2 diet, a second clutch of eggs was collected when the phase-2 diet was different from the phase-1 diet of either test fish.

Phase 4. The 7-d survival and growth of fathead minnow progeny were determined. After hatching, 10 embryos were transferred to a 1-L beaker (Figure 1). When a clutch had 30 or more embryos, three beakers with 10 embryos each were used. The beakers were maintained at 25 ± 1 °C with a photoperiod of 16 h light:8 h dark. Larvae were fed newly hatched brine shrimp nauplii three times daily until day 7, when they were sacrificed, dried in an oven at 65 °C for 24 h, and weighed (± 0.1 mg).

Reproductive Endpoints. Multiple endpoints of reproductive success were measured, including (1) gonadal development of females and males, (2) spawning success, (3) days to spawning, (4) reproductive effort of female fish, (5) developmental success of embryos, (6) hatching success of embryos, (7) survival of larvae, and (8) growth of larvae. *Gonadal development* was quantified for each adult fish, whether or not it spawned, as the gonadosomatic index (GSI), the percentage of the whole-body weight contributed by the gonads. *Spawning success* was the percentage of pairs within a dietary treatment that spawned a clutch of eggs within 21 d after placement in the breeding aquaria. A clutch of eggs was defined a priori as more than five eggs oviposited between daily examinations of a spawning substrate. *Days to spawning* was the number of days elapsed before a pair of fish spawned a clutch of eggs. Although a clutch of five eggs was used as the criterion for days to spawning, pairs of fish were kept in phase-2 aquaria until they spawned 30 or more eggs, a number sufficient for measurement of the other reproductive variables (e.g., hatching success). Reproductive effort of female fish was the total number of eggs laid (until spawning ≥ 30 eggs) per gram of fish, a ratio that accounts for the biomass of the female from which energy and nutrients are allocated for the production of eggs (*21*). *Daily reproductive effort* (i.e., eggs g^{-1} d^{-1}) was estimated by normalizing reproductive effort to the number of days a female was kept in a phase-2 aquarium. *Developmental success* was the percentage of embryos in each clutch that reached the "eyed" stage. *Hatching success* was the percentage of "eyed" eggs

that hatched. *Survival of larvae* was the percentage of the initial number of hatched larvae that was surviving in a beaker on day 7 (post hatch). *Growth of larvae* was the mean dry weight of surviving larvae on day 7. Breeding pairs were sacrificed on the day that they spawned more than a cumulative total of 30 eggs or after 21 d if they did not spawn. Each adult fish was placed in a food-grade plastic bag, anesthetized in a refrigerator, blotted dry, measured (± 1 mm total length), weighed (± 0.1 mg), and dissected to remove the gonads, which were also weighed (± 0.1 mg). Carcasses (i.e., whole fish minus gonads) were promptly frozen at ≤ −30 °C until lyophilization.

We took steps to minimize contamination of fish carcasses during dissection. Fish were dissected with stainless steel implements on the inner surface of a food-grade plastic bag inside a laminar-flow hood. Between fish, dissection equipment was rigorously cleaned with detergent and rinsed with reagent-grade water, dissection surfaces were changed, and gloves were cleaned or changed.

MeHg-Contaminated Diets. Phase-1 and phase-2 diets were prepared by mixing fish food (Soft-moist fish food, Nelson and Sons, Inc.) with reagent alcohol (Fisher) containing dissolved methylmercuric chloride (Alfa Chemical). Control diets were prepared similarly by mixing fish food with alcohol only. Alcohol was evaporated from the mixtures in acid-cleaned, glass pans in a fume hood. Diets were prepared about every 2 weeks and frozen until use. Samples of each diet from each preparation batch were analyzed for total mercury. The mean caloric density of diets, determined by bomb calorimetry, was 5.16 Kcal g^{-1} dry weight (relative standard deviation, 0.5%).

Estimated Exposure to Waterborne MeHg. We did not measure MeHg in aquarium water but did examine dissociation of MeHg from contaminated food in well water to estimate the associated potential exposure of fathead minnows to waterborne MeHg. Samples of each diet were soaked in well water for 10 min or 22 h and collected on acid-cleaned Gelman Type A/E filters. Both diet sample and filter were acid-digested and analyzed for total mercury.

Little MeHg dissociated from contaminated diets in well water. Samples of contaminated diets that had soaked for 10 min lost from 2.7% to 5.2% of the mercury measured in unsoaked samples. Loss of MeHg from contaminated diets soaked for 22 h ranged from 7.6% to 10%. Total mercury concentrations in soaked samples of the control diet were similar to those in unsoaked samples.

Potential concentrations of MeHg in culture water were estimated from (1) the amount of food put into the tanks daily, (2) the approximate amount of food consumed, (3) the dissociation of MeHg from the diets, and (4) the volume and rate of water replacement in the tanks. Fathead minnows in the 500-L tanks (phase 1) were fed intermittently a maximum of 36 g of food per day, more than half of which was consumed within 10 min after each feeding. Based on the volume and average inflow of water to the tanks (30 L h^{-1}), about 80% of the tank volume was replaced every 24 h (*22*). We estimate that maximal levels of waterborne MeHg could have ranged from 1 ng L^{-1} in the low-MeHg tank to 8 ng L^{-1} in the high-MeHg tank, assuming that (1) half of the food was consumed, (2) the inflowing water contained no MeHg, (3) 10% of the MeHg dissociated from uneaten food during a 24-h period, and (4) all of the dissociated MeHg remained dissolved. These MeHg concentrations are $10-10^2$ fold greater than those in oxic waters of low-alkalinity lakes in northern Wisconsin (0.05–0.33 ng Hg L^{-1} (*23*)) but are considerably (10^2-10^3 fold) less than the waterborne concentrations known to affect the survival, development, reproduction, physiology, and behavior of juvenile and adult fishes (*2*). Moreover, the concentrations of MeHg in our contaminated diets exceeded the estimated maximal concentrations in water by about 10^6. Thus, it can be reasonably inferred that the effects of MeHg on reproduction of fathead minnows in this study resulted from dietary exposure.

Mercury Determinations. Diets and carcasses of adult fish were analyzed individually for total mercury. Frozen carcasses were lyophilized to a constant dry weight in food-grade plastic bags for 102−168 h at ≤ −50 °C. Lyophilized carcasses weighing 0.5 g or more were pulverized and homogenized inside their plastic bags. We acid-digested whole, lyophilized carcasses of fish weighing less than 0.5 g, 0.4-g subsamples of lyophilized carcasses weighing ≥ 0.5 g, and 0.25-g subsamples of diets following the methods of Hammerschmidt et al. (*6*). Each digestate was analyzed by flow injection cold-vapor atomic absorption spectroscopy with a Perkin-Elmer FIMS 100.

Quality Assurance. All equipment used to culture fathead minnows was acid cleaned and rinsed with either reagent-grade water (nominal resistance ≥ 15 MΩ cm^{-1}) or well water. For determinations of total mercury, glassware and equipment were acid cleaned and rinsed with reagent-grade water. All acids and reagents used in digestions and analyses were suitable for use in mercury determinations (J. T. Baker). Mercury standards were prepared from a 1000-mg L^{-1} certified standard (J. T. Baker).

Accuracy of determinations of total mercury for each analytical batch of samples was quantified by analyses of (1) certified reference materials from the National Research Council of Canada and the U.S. National Institute of Standards and Technology, (2) replicate subsamples of homogenized fish and test diets, (3) spiked (before digestion) subsamples of homogenized fish and diets, and (4) blanks and standards taken through the digestion procedures. Mean measured concentrations and 95% confidence intervals (CI) of total mercury in the four reference materials analyzed were within the certified ranges, which ranged from 0.27–0.39 μg g^{-1} to 4.38−4.90 μg g^{-1} dry weight. Method precision (relative standard deviation) for determinations of total mercury, estimated from analyses of duplicate and triplicate subsamples, averaged 4.7% (range, 0.2−17.9%) for fish and 4.1% (range, 1.3−9.1%) for diets. Mean recovery of total mercury was 98% (95% CI, 97−100%) for 111 spiked subsamples of fish and 96% (CI, 93−100%) for 36 spiked subsamples of diets. Our estimated method detection limit (*24*) for total mercury in a 0.25-g sample of homogenized fish was 0.004 μg Hg g^{-1} dry weight.

Statistical Analyses. Data were analyzed with a microcomputer and SPSS for Windows software (version 8.0). Least-squares linear regression models were used to describe relations between (1) measures of reproductive success and total mercury in fish carcasses and (2) the different reproductive variables measured. Spawning success, developmental and hatching success of embryos, and survival and growth of larvae were examined only for eggs and progeny from the 100 pairs of adult fish for which both parents were fed the same diet during each phase throughout the study, although the diet of both fish may have changed from one phase to another (e.g., male and female fed the medium-MeHg diet during phase 1 and the control diet during phase 2). Only data from the 50 pairs of fish fed the same diet during phases 1 and 2 (e.g., male and female fed medium diet during both phase 1 and 2) were used in regression analyses of days to spawning. All spawning females ($n = 52$) and males ($n = 47$) fed a single diet during both phases 1 and 2, regardless of the exposure of their mate, were used in statistical analyses of the effects of MeHg on gonadal development. Measurements from all spawning females ($n = 52$) fed the same diet during both phases 1 and 2 were used to assess the effects of MeHg on reproductive effort. For measures of reproductive success that were related to parental exposure to dietary MeHg, a Wilcoxon rank sum test was

TABLE 2. Wet Weight, Spawning Success, and Total Mercury in Carcasses of Male and Female Fathead Minnows Fed Diets with Different Levels of Methylmercury[b]

diet	mean wet weight (g)		n^a	spawning success (%)	burden (μg Hg fish^{-1})		concentration (μg Hg g^{-1} dry wt)	
	male	female			male	female	male	female
Same Diet during Phases 1 and 2								
control	4.44 (2.94–6.83)	1.89 (1.48–2.48)	16	75	0.36 (0.18–0.56)	0.21 (0.11–0.43)	0.32 (0.20–0.43)	0.48 (0.27–0.43)
low	3.70 (2.78–4.26)	1.94 (1.16–2.57)	13	46	1.51 (0.66–2.11)	1.51 (0.66–2.11)	2.83 (2.13–3.87)	3.40 (2.23–4.54)
medium	4.79 (2.51–6.14)	2.12 (1.73–2.74)	10	50	13.5 (9.00–1.6)	7.4 (5.15–9.54)	11.7 (9.98–13.8)	14.0 (9.78–20.9)
high	5.12 (3.81–8.54)	2.50 (1.33–3.67)	11	36	25.5 (18.9–38.2)	14.2 (7.40–22.2)	18.4 (14.8–25.0)	22.2 (15.1–26.4)
Contaminated Diet during Phase 1 and Control Diet during Phase 2								
low	4.26 (3.28–5.40)	1.83 (1.39–2.25)	8	63	2.02 (1.69–2.27)	1.07 (0.84–1.23)	1.97 (1.38–2.61)	2.59 (1.95–3.57)
medium	4.35 (3.51–5.47)	2.29 (1.70–2.76)	10	40	7.05 (3.99–9.68)	4.17 (3.30–5.45)	6.64 (2.50–10.4)	7.51 (5.88–9.70)
high	4.51 (3.58–6.20)	2.27 (1.66–3.30)	7	14	14.8 (8.50–20.3)	6.93 (4.06–9.17)	11.6 (10.3–13.4)	12.1 (9.18–17.6)
Control Diet during Phase 1 and Contaminated Diet during Phase 2								
low	4.38 (3.36–6.42)	2.07 (1.18–2.91)	11	55	1.06 (0.40–1.91)	0.69 (0.25–1.44)	1.02 (0.45–2.29)	1.41 (0.52–2.40)
medium	3.39 (2.34–3.94)	1.79 (1.49–2.28)	7	86	4.03 (1.73–6.74)	2.66 (1.09–4.65)	4.92 (2.51–8.80)	6.57 (2.90–11.8)
high	4.89 (3.67–6.83)	1.99 (1.41–2.53)	7	100	8.36 (2.64–19.7)	5.17 (1.46–10.1)	6.48 (2.34–11.2)	11.2 (2.69–21.6)

[a] Number of pairs of fathead minnows examined. [b] Ranges are given in parentheses.

used to contrast the relative effect of either male or female exposure to MeHg.

The number of days until each pair of fish spawned a clutch of eggs may have exceeded the period of time that the fish were in the breeding quadrants (21 d), and, therefore, reproduction went unobserved. These data are said to be right-censored (*25*), and the statistical analysis must account for the censoring. We used a Cox regression model for time-dependent variables (*25*) to assess the effect of MeHg in females on days to spawning of the 50 pairs of fish fed the same diet during both phases 1 and 2. This is an extension of the Cox proportional hazards model of the distributions of times until a single event, such as mortality. The instantaneous rate of reproduction generated by the regression model, denoted by the hazard function $h(t)$, is an estimate of the potential for reproduction per unit time at a particular instant, given that reproduction has not already occurred and can be defined as

$$h(t) = [h_0(t)] \exp[\beta C + \delta C \times t] \quad (1)$$

where $h_0(t)$ is an unknown baseline hazard function, β and δ are regression coefficients, C is the mercury concentration of the female carcass (μg Hg g^{-1} dry weight), and t (time) ranged from 1 to 21 d. Therefore, the potential for spawning by a pair of fathead minnows, given that they have not already spawned, is a function of some baseline reproduction rate; the potential is increased (exp term > 1) or decreased (exp term < 1) by mercury in the female and is not constant with time.

The ratio of the potential for reproduction between two groups of fathead minnows with different mercury concentrations can be calculated as

$$\text{relative reproductive potential} = \exp[\beta(C_1 - C_2) + \delta((C_1 \times t) - (C_2 \times t))] \quad (2)$$

where C_1 and C_2 are the mean mercury concentrations in female fathead minnows of the two groups. We fitted eq 1 to the reproduction data (i.e., days to spawning) by maximizing the partial likelihood and constructed Wald χ-square tests for each parameter (*25*).

Results and Discussion

Dietary MeHg did not reduce the growth and survival of adult fathead minnows in our study. There was considerable overlap in wet weights of fathead minnows of each sex among the four phase-1 diets (Table 2). Mean wet weight ranged from 3.70 g (low MeHg) to 5.12 g (high MeHg) in males and from 1.89 g (controls) to 2.50 g (high MeHg) in females. Wet weights of spawning and nonspawning fish were positively related to mercury burdens for all males ($r^2 = 0.19$, $p < 0.001$, $n = 179$) and females ($r^2 = 0.23$, $p < 0.001$, $n = 188$) combined, suggesting that exposure to dietary MeHg, at levels that were not overtly toxic to the fish, stimulated somatic growth (i.e., hormesis). Mortality of adult fish was small and unrelated to dietary MeHg, ranging from 0.07% for fish fed the low-MeHg diet to about 0.4% for fish fed the control and high-MeHg diets during phase 1. Only one fish died during phase 2, a male fed the medium-MeHg diet during both phases 1 and 2. These results were unexpected, given previous investigations showing decreased survival and growth of adult and juvenile fishes exposed to dietary MeHg (*11*, *26*, *27*).

Concentrations of mercury were notably higher in female fathead minnows than in males fed the same diet (Table 2), probably because female fish consume more food than males to support the energy requirements of egg production (*28*, *29*). The increased feeding rates in females cause greater dietary uptake of methylmercury, and only a small fraction of the accumulated methylmercury is transferred to the egg mass and eliminated during spawning (*6*, *7*, *13*).

Dietary MeHg reduced gonadal development of female fathead minnows. The GSI of spawning females fed the same diet during phases 1 and 2 ranged from 3.0% to 16.4% and was related inversely to the concentration of total mercury in carcasses ($r^2 = 0.15$, $p = 0.005$, $n = 52$). The GSI of females fed a contaminated diet during phase 1 and the control diet during phase 2 was not correlated with total mercury in carcasses ($p = 0.54$, $n = 25$). Likewise, the GSI of females fed the control diet during phase 1 and a contaminated diet during phase 2 was not correlated with total mercury in carcasses ($p = 0.65$, $n = 25$), even though fish fed contaminated diets during phase 2 readily accumulated MeHg (Table 2). Insufficient samples sizes may have precluded detection of the effects of mercury on GSI when diets differed between phases 1 and 2.

Dietary MeHg also reduced the daily reproductive effort of female fathead minnows (Figure 2). The average daily number of eggs laid per gram of female carcass was highly variable but was correlated negatively with concentrations of total mercury in carcasses of females fed the same diet during both phases 1 and 2 ($r^2 = 0.14$, $p = 0.01$, $n = 46$). The total number of eggs laid per gram of carcass was correlated positively with GSI ($r^2 = 0.21$, $p = 0.001$); hence, dietary MeHg reduced gonadal development of female fish, and this effect was linked to lower egg production. Exposure of female fish to MeHg can interfere with the production of estrogen, thereby reducing the number, size, or quality of eggs

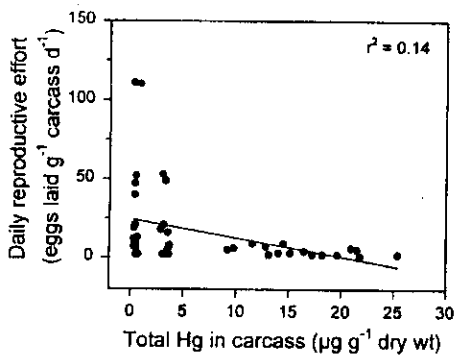

FIGURE 2. Relation between reproductive effort and concentration of total mercury in carcasses of female fathead minnows fed the same diet during phases 1 and 2.

produced. Estrogen stimulates the liver to produce vitellogenin, a macromolecule that is a substrate for developing eggs (*30*). Intraperitoneal exposure to MeHg depressed estrogen levels in female tilapia *Oreochromis niloticus* (*31*), and prolonged exposure to a high concentration of aqueous MeHg interfered with vitellogenesis in catfish *Clarias batrachus* (*8*).

In male fathead minnows fed the same diet during phases 1 and 2, the GSI ranged from 0.4% to 2.3% and was unrelated to total mercury in carcasses. The GSI may not, however, be a sensitive measure of gonadal development in male fish exposed to MeHg. For example, the GSI of male walleye *Stizostedion vitreum* fed a MeHg-contaminated diet containing about 1.0 μg Hg g^{-1} wet weight did not differ from that of controls, but histological examination showed disruption of the normal architecture of the testes (*11*). Hence, gonadal development of male fish exposed to MeHg might be better evaluated by histological examination than with GSI (*11*).

Dietary MeHg reduced the spawning success of fathead minnows (Table 2). Spawning success of pairs fed the control diet during phases 1 and 2 was 75% (i.e., 12 of 16 pairs spawned). Although fish fed contaminated diets were generally larger than those fed the control diet, their spawning success was less: 46%, 50%, and 36%, respectively, for pairs fed the low-, medium-, and high-MeHg diets during both phases 1 and 2.

The influence of dietary MeHg on spawning apparently was in response to phase 1 exposure (i.e., as juveniles), given that spawning success of contaminated fish was not improved by a shift to the control diet during phase 2 (Table 2). Spawning success was 63%, 40%, and 14%, respectively, for pairs fed the low-, medium-, and high-MeHg diets during phase 1 and the control diet during phase 2. After spawning their final clutch of eggs, mean concentrations and burdens of total mercury in carcasses of contaminated fish fed the control diet during phase 2 were less than those in fish fed a contaminated diet during both phases 1 and 2 (Table 2).

Interestingly, exposure to dietary MeHg during phase 2 did not reduce the spawning success of fish fed the control diet during phase 1 (Table 2). Spawning success was 55%, 86%, and 100% for pairs fed the low-, medium-, and high-MeHg diets during phase 2. Concentrations and burdens of mercury were clearly elevated in fish fed the medium- and high-MeHg diets during phase 2, yet their spawning success exceeded that of pairs fed the control diet during both phases 1 and 2 (75%). A reason for enhanced spawning success of these fish is unknown, but the mechanism by which phase-2 dietary MeHg elicited this effect likely is neurological (e.g., perception of mate) rather than hormonal (e.g., heightened gonadal maturation), given that the fish were sexually mature when placed into the breeding aquaria.

Spawning success of pairs was similar whether only males or only females were exposed to dietary MeHg during phase 1. Regardless of the diet in phase 2, the spawning success of pairs with females fed any of the three contaminated diets and males fed the control diet during phase 1 was 40%, similar to that of pairs with females fed the control diet and males fed a contaminated diet (51%; Fisher's exact test, $p = 0.397$).

Spawning success of fathead minnows was related to gonadal development of female fish. For females fed the same diet during both phases 1 and 2, the mean GSI of spawning fish was greater than the GSI of fish that failed to spawn within 21 d (Wilcoxon rank sum test, $p < 0.001$), averaging 9.1% and 6.3% for spawning and nonspawning females fed the control diet, 11.1% and 6.3% for fish fed the low-MeHg diet, 8.2% and 4.5% for fish fed the medium-MeHg diet, and 6.2% and 5.2% for fish fed the high-MeHg diet. Thus, reduced gonadal development of female fathead minnows, an effect elicited by dietary MeHg in spawning females, diminishes the capacity of individual fish to spawn, subsequently reducing the spawning success of the population.

Concentrations of total mercury in fathead minnows fed the low-MeHg diet (Table 2) were similar to those in some piscivorous fish in low-alkalinity lakes and newly flooded reservoirs having elevated MeHg in food webs (*2*, *32*–*34*). Relative to controls, the spawning success of our test fish was reduced by dietary concentrations of MeHg of 0.17 μg Hg Kcal^{-1} (low-MeHg diet) or greater, a concentration equaled or exceeded in the prey of some piscivorous and invertivorous fish in low-alkalinity lakes and flooded reservoirs (Table 1). Moreover, Paterson et al. (*35*) found MeHg in zooplankton as high as 1.09 μg Hg g^{-1} dry weight (about 0.21 μg Hg Kcal^{-1}) after flooding of a peatland reservoir in northwestern Ontario, suggesting that even zooplanktivorous fish could be exposed to dietary MeHg high enough to alter reproduction in aquatic systems with very high rates of MeHg production.

MeHg also delayed spawning of fathead minnows. Days to spawning was correlated positively with the concentration of total mercury in carcasses of both females ($r^2 = 0.33$, $p = 0.002$) and males ($r^2 = 0.33$, $p = 0.002$) fed the same diet during both phases 1 and 2. In females, days to spawning also was related inversely to the GSI ($r^2 = 0.22$, $p = 0.014$). Days to spawning of pairs with females fed a contaminated diet and males fed the control diet during phase 1 averaged 10 d, exceeding that for pairs with females fed the control diet and males fed a contaminated diet (mean, 7 d; Wilcoxon rank sum test, $p = 0.051$).

MeHg decreased the instantaneous rate of reproduction for female fathead minnows fed the same diet during both phases 1 and 2 (β [SE] = −0.126 [0.048], Wald $\chi^2 = 7.02$, df = 1, $p = 0.008$), and the effect changed with time (δ [SE] = 0.009 (0.005), Wald $\chi^2 = 4.00$, df = 1, $p = 0.045$). Consequently, on day 1 the relative reproductive potential of female fathead minnows with total mercury in carcasses averaging 0.48 μg Hg g^{-1} dry weight (control diet; Table 2) was 1.4 times that for fish averaging 3.4 μg Hg g^{-1} dry weight (low-MeHg diet), 4.9 times that for fish averaging 14 μg Hg g^{-1} (medium-MeHg diet), and 12.7 times that for fish averaging 22 μg Hg g^{-1} (high-MeHg diet).

Complete inhibition of reproduction has obvious consequences, but delayed reproductive activity also can reduce survival and recruitment in fish populations. Fathead minnows are almost in reproductive condition when they become sexually dimorphic; therefore, our test fish were presumably capable or nearly capable of spawning when they were placed into the breeding aquaria. However, many pairs of phenotypically mature fish fed a contaminated diet during phase 1 did not spawn within 21 d, whereas 92% of the pairs fed the control diet during both phases 1 and 2 spawned within 6 d. Under natural conditions, many fishes reproduce for only a brief period in late spring or early summer. Delayed

TABLE 3. Developmental and Hatching Success of Embryos and Survival and Weight of Larval Fathead Minnows Measured 7 d after Hatching[a]

	mean (range)			
diet	developmental success (%)	hatching success (%)	larval survival (%)	mass of individual larvae (mg)
Same Diet during Phases 1 and 2				
control	60 (0–100)	91 (65–100)	78 (0–100)	0.6 (0.2–0.9)
low	54 (2–100)	76 (36–100)	85 (30–100)	0.9 (0.5–1.3)
medium	73 (54–93)	59 (6–99)	79 (57–96)	0.7 (0.7–0.8)
high	43 (21–72)	99 (96–100)	85 (80–90)	0.6
Contaminated Diet during Phase 1 and Control Diet during Phase 2				
low	43 (11–90)	50 (0–93)	90 (65–100)	0.6 (0.2–1.0)
medium	65 (0–96)	90 (79–100)	88 (83–93)	0.8 (0.6–0.9)
high	0			
Control Diet during Phase 1 and Contaminated Diet during Phase 2				
low	50 (0–88)	89 (78–96)	93 (90–97)	0.8 (0.7–0.9)
medium	60 (0–100)	91 (83–100)	89 (85–97)	0.7 (0.5–1.2)
high	49 (0–91)	74 (4–100)	69 (0–95)	0.7 (0.5–0.9)

[a] Parental fish were fed one of four diets during phases 1 and 2.

spawning can reduce the survival of young fishes by disrupting the timing of endogenous to exogenous feeding relative to seasonally abundant food resources, such as plankton and small prey fish. Failure of young fish to exploit such resources can result in decreased growth—a factor that can increase the susceptibility of young-of-the-year fish to predation (*36*) and reduce their over-winter survival (*37*).

Developmental and hatching success of embryos and 7-d survival and weight of larvae varied considerably in our study (Table 3) but were not correlated with concentrations of mercury in either the diets or carcasses of parental fish. No significant effect on either fertilization success, hatching success, or larval survival also was observed when adult killifish *Fundulus heteroclitus* were exposed to similar dietary levels of MeHg and contained similar whole-body concentrations of mercury (*38*). Sample size in our study may have been too small to detect effects of MeHg on these variables, given their high variation within dietary test groups. For these measures, we examined 100 pairs of fish; of these, only about half spawned and fewer produced viable embryos, and even fewer produced larvae. In addition, an increased duration of observation for larval survival and growth, far beyond 7 d post-hatch, could have been more effective for identifying effects of maternally transferred MeHg (*38, 39*). Fjeld et al. (*40*) for example, observed impaired feeding efficiency and reduced competitive ability in 3-year-old grayling (*Thymallus thymallus*) that had been exposed as eggs to waterborne MeHg for 10 d during embryogenesis.

Latif et al. (*3*) examined the effects of both maternally transferred and waterborne MeHg on embryos and larvae of walleye from industrially polluted Clay Lake and two remote lakes in Manitoba. In their study, the hatching success of eggs and heart rate of embryos decreased with increasing, environmentally relevant concentrations of waterborne MeHg (range, 0.1–7.8 ng L^{-1}), whereas MeHg in eggs from maternal transfer did not affect either hatching success or heart rate. The growth of larval walleye, measured 8 d after hatching, was not affected by either maternal or waterborne MeHg (*3*).

Dietary MeHg affected the overall reproductive performance of adult fathead minnows but maternally transferred MeHg did not measurably affect the embryos and larvae produced. Previous studies (*12*) have shown that MeHg reduces the survival and growth of embryo and larval fish, yet few have examined reproductive effects of MeHg on mature fishes, a life stage that is generally less sensitive to MeHg (*2*). In our study, dietary MeHg reduced gonadal development of female fathead minnows, which may explain the reduced proportion of spawning fish, delayed spawning, and low reproductive effort of contaminated pairs of fish. Any of these reproductive effects alone can have serious consequences for the survival and recruitment of fish populations. Yet, we observed a cumulative sequence of effects in fish exposed to dietary MeHg. Moreover, the concentrations of dietary MeHg that adversely affected reproductive success of the fathead minnows are encountered in aquatic food webs of many surface waters.

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Effects of Dietary Methylmercury on Reproductive Endocrinology of Fathead Minnows

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Recent laboratory studies have demonstrated that environmentally realistic concentrations of dietary methylmercury can impair reproduction of fish. To evaluate relations between reproductive success and biomarkers of methylmercury exposure, we fed juvenile fathead minnows (*Pimephales promelas*) one of three diets contaminated with methylmercury: 0.06 (control), 0.87 (low), and 3.93 (medium) μg of Hg g^{-1} dry weight. At sexual maturity, fish were paired, allowed to reproduce, and then analyzed for total mercury, plasma testosterone (T), and 17β-estradiol (E2). Diets did not affect survival or growth of fathead minnows. Methylmercury suppressed levels of T in males and E2 in females. Male fathead minnows fed the control diet had mean T concentrations 20% and 106% greater than those fed the low and medium diets; control females had mean E2 concentrations 149% and 402% greater than those fed the low and medium diets. Methylmercury also inhibited gonadal development of females; the gonadosomatic index (GSI) of females fed the medium diet was 40% less than that of females fed control or low diets. Plasma levels of T in males and E2 in females were positively related to GSI. Methylmercury reduced the reproductive success of fathead minnows. Spawning success was 32% for pairs fed the control diet, 12% for pairs fed the low diet, and 0% for pairs fed the medium diet. Pairs fed the low diet required, on average, 5 d longer to spawn a clutch of eggs than the controls. Concentrations of methylmercury fed to fathead minnows in this study are also encountered by invertivorous and piscivorous fish in some methylmercury-contaminated aquatic ecosystems. This suggests that reproduction of wild fishes may be adversely affected by methylmercury and that suppressed hormone levels may be used to indicate diminished reproduction of fish.

Introduction

Few studies have demonstrated a direct relation between exposure to contaminants and subsequent adverse effects on wild populations of fish. Difficulties in documenting effects of contaminant exposure arise from natural fluctuations in population demographics as well as synergistic and cumulative effects of natural and other anthropogenic stressors (*1*). Recent field studies investigating effects of endocrine-disrupting chemicals on reproduction of wild populations of fish have emphasized the use of reproductive biomarkers to provide quantifiable measures of molecular and physiological change (e.g., refs *2* and *3*). Reproductive biomarkers can include induced production of the yolk-precursor vitellogenin in males, circulating levels of sex hormones such as testosterone (T) and 17β-estradiol (E2), indices of gonadal status, and expression of secondary sex characteristics. Little evidence exists, however, to indicate that alterations in reproductive biomarkers result in population-level effects. For example, effluents containing environmental estrogens in rivers of the U.K. (*4*) and United States (*5*) cause widespread disruption in gonadal development and alteration of sex hormone profiles in cyprinid fishes, yet effects on fish recruitment have not been detected. Arcand-Hoy and Benson (*6*) stress the importance of establishing relations between reproductive biomarkers and effects on populations (e.g., reproductive success) through the use of experimental laboratory studies.

Mercury contamination of the environment is a problem of global concern. Globally increasing concentrations of atmospheric mercury from anthropogenic sources have led to increased mercury deposition (*7, 8*), which is the principal source of mercury for many surface waters (*9*). In the aquatic environment, bacteria can transform inorganic mercury into methylmercury (MeHg; *10*), which is highly toxic and readily bioaccumulates in exposed organisms, biomagnifying in aquatic food webs (*11, 12*). The ecological implications of MeHg in aquatic food webs have focused primarily on health and reproductive risks associated with consumption of contaminated fish tissue by humans and piscivorous birds and mammals. To date, few studies have focused on the population-level effects of MeHg on the fish themselves (*13*).

Methylmercury can disrupt endocrine function of fish. Arnold (*14*) reported gonadal lesions and abnormal levels of 11-ketotestosterone and E2 in laboratory fish exposed to MeHg. Moreover, elevated concentrations of MeHg in aquatic food webs may adversely affect reproduction of some wild fish. Levels of dietary MeHg that reduced reproductive success of fathead minnows (*Pimephales promelas*; *15*) and killifish (*Fundulus heteroclitus*; *16*) in laboratory studies are also encountered by fish in some surface waters. The relation has not been established, however, between reproductive biomarkers and reproduction altered by MeHg.

The primary objective of this research was to determine relations among concentrations of plasma T and E2, gonad weight, and reproductive success of fish exposed to dietary MeHg. We fed juvenile fathead minnows diets contaminated with concentrations of MeHg encountered by some fish in the environment, maintained the fish through sexual maturity, and evaluated the effects of dietary MeHg on endocrine function and reproductive success.

Experimental Section

Fish. Fathead minnows were obtained as embryos from the Upper Midwest Environmental Sciences Center (U.S. Geological Survey, La Crosse, WI) and raised to sexual maturity. Embryos were collected from outdoor ponds and placed in a single hatching jar with flowing water in the laboratory. Hatched larvae were then allowed to swim out into two 500-L flow-through aquaria. Larvae were fed brine shrimp (*Artemia nauplii*) for 60 d and gradually acclimated to a diet of Sterling Silver Cup Fish Food (Nelson and Sons, Inc., Murray, UT) for 30 d. Ninety days after hatching, 200 fathead minnows were transferred to each of fifteen 180-L flow-through aquaria.

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TABLE 1. Mercury Concentrations in Diets Fed To Test Fish in This Study in Comparison to Concentrations of Mercury in Zooplankton, Benthic Invertebrates, and Forage Fish in Low-Alkalinity Lakes and Newly Flooded Reservoirs[a]

item	Hg concn (μg g^{-1} dw)	ref
control diet	0.06	this study
zooplankton	0.06–0.28	*11*
benthic invertebrates	<0.01–1.02	*12*
low diet	0.87	this study
whole yellow perch	0.12–1.16	*47*
rainbow smelt	0.20–2.28	*54*
crayfish	0.84–2.46	*55*
medium diet	3.93	this study

[a] Adapted from ref *15*.

Fish were exposed to MeHg in these aquaria until sexual maturity.

Fish were cultured according to standard methods for toxicity testing (*17, 18*). Fathead minnows were maintained in aquaria receiving well water with a 16:8-h light:dark cycle. Water quality characteristics were measured daily (temperature 23.6 ± 0.1 °C, dissolved oxygen 6.70 ± 0.01 mg L^{-1}) or weekly (alkalinity 300 ± 2 mg L^{-1} as $CaCO_3$, pH 8.06 ± 0.01, hardness 348 ± 3 mg L^{-1} as $CaCO_3$, ammonia 0.2 ± 0.0 mg L^{-1} as NH_3-N; *19*). Deposited and suspended wastes were siphoned daily from each aquarium, and residue on aquaria was removed weekly by scraping. All aquaria and equipment were acid-cleaned before use; separate cleaning equipment was used for each aquarium to avoid possible transmission of pathogens.

MeHg Exposure. Fathead minnows were fed MeHg-contaminated food (5% of body mass per day) to increase burdens of MeHg in fish. Nearly all (95–99%) mercury in fish is MeHg (*20*), and it accumulates almost entirely via dietary uptake (*21*). Fish were fed a contaminated diet with one of three concentrations of total mercury (mean ± 1 SE): 0.058 ± 0.004 (control), 0.87 ± 0.02 (low MeHg), and 3.93 ± 0.08 (medium MeHg) μg g^{-1} dry weight (dw). These concentrations approximate MeHg concentrations in the diets of invertivorous and piscivorous fish from midcontinental low-alkalinity lakes (Table 1) and replicate the diets (control, low, medium) fed to fathead minnows by Hammerschmidt et al. (*15*). Each 180-L aquarium was randomly assigned one of the three diets to yield five aquaria per dietary treatment. Fish were fed MeHg-contaminated food for about 250 d (from 90 d post-hatch until termination of experiment).

Contaminated diets were prepared by mixing fish food with reagent alcohol containing dissolved methylmercuric chloride (*15*). Control diets were prepared by mixing fish food with alcohol only. Alcohol was evaporated overnight from the fish food in acid-washed glass pans in a fume hood. Diets were prepared as needed and frozen until use. Samples from each batch were analyzed for total mercury.

Reproductive Tests. Adult fathead minnows display strong sexual dimorphism during breeding. Sexual maturity of males is denoted by the development of spawning color, rostral tubercles, and a soft mucus-secreting dorsal fat pad (*17*). Females exhibit an extended urogenital papilla when mature (*22*).

After fathead minnows became sexually dimorphic (approximately 300–320 d after hatching), five breeding pairs from each 180-L aquarium were selected and randomly assigned to quadrants, within treatment, to one of fifteen 50-L breeding aquaria receiving well water. Fish in the 50-L breeding aquaria received the same diet they were fed in the 180-L aquaria. One acid-washed spawning substrate (a half cylinder of PVC pipe) was placed into each quadrant and examined daily for eggs.

End points of reproductive success measured in fish included spawning success, days to spawning, and relative fecundity of female fish. Spawning success was the percentage of pairs within a dietary treatment that spawned a clutch of eggs within 21 d after placement in breeding aquaria. A clutch of eggs was defined a priori as six or more eggs. Days to spawning was the number of days required for a pair of fish to spawn a clutch of eggs. Relative fecundity of female fish was calculated as the number of eggs laid per gram of fish. Days to spawning and relative fecundity of female fish were measured only for pairs that spawned a clutch of eggs.

Sample Collection. After spawning or after 21 d if no spawning occurred, adult fathead minnows were euthanized with an excess of MS-222 (Sigma-Aldrich). Blood was collected from the caudal vein in heparinized hematocrit capillary tubes (Fisher Scientific) by severing the caudal peduncle. Because hormone levels in fish may vary diurnally, blood samples were collected from all fish between 0900 and 1200 h and were centrifuged for 10 min at 4 °C. Plasma was drawn from the capillary tubes and stored at −80 °C until analysis of sex hormones. The weight (± 0.1 mg), total length (± 1 mm), and sex of each fish were recorded. Gonads were dissected from the fish and weighed (± 0.1 mg). To minimize mercury contamination of carcasses, fish were dissected inside a laminar-flow hood with soap-washed stainless steel scalpels on the inner surface of separate food-grade plastic bags. Carcasses were frozen (−30 °C) until lyophilization and analysis of total mercury.

Mercury Determination. Diets and lyophilized carcasses of adult fathead minnows were analyzed for total mercury by cold-vapor atomic absorption spectroscopy. Lyophilized carcasses weighing less than 0.5 g, 0.4-g subsamples of lyophilized carcasses weighing 0.5 g or greater, and 0.25-g subsamples of diets were acid-digested (*23*) and analyzed with a Perkin-Elmer FIMS 100. Glassware used in analyses were acid-cleaned and rinsed with reagent-grade water (nominal resistance > 15 MΩ cm^{-1}). Acids and reagents used in digestions and analyses were suitable for trace metal analysis (J. T. Baker).

The accuracy of mercury determinations for each analytical batch was determined by analyses of (i) certified reference materials (National Research Council of Canada), (ii) triplicate subsamples of homogenized fish and diets, (iii) spiked samples of homogenized fish and diets, and (iv) procedural blanks and calibration standards. Mean measured concentrations of total mercury in the three reference materials analyzed were within certified ranges, which ranged from 0.21–0.33 to 4.38–4.90 μg g^{-1} dw. Method precision (relative standard deviation) of mercury determinations, estimated from triplicate analyses of fish and diets, averaged 4.0%. Mean recovery of mercury from spiked samples was 98%. The method detection limit (*19*) for total mercury in a 0.25-g sample of fish food was 0.001 μg g^{-1} dw.

Reproductive Biomarkers. Plasma T and E2 were quantified by competitive enzyme immunoassay with Cayman Chemical EIA kits. Assay procedures used were specified by the manufacturer (*24, 25*), with two exceptions: (i) due to small sample volume, reagent and sample volumes used in the assays were reduced by half (i.e., to 25 μL of plasma) and (ii) standard curves were established with linear regression by plotting absorbance units as a function of analyte concentration (*26*). Hormones were extracted from plasma with ether before analysis. A 5-mL aliquot of diethyl ether was added to each plasma sample in a glass tube. Each tube was vortexed for 60 s, and the ether layer was transferred to a clean tube with a pasteur pipet. This process was repeated twice with 3-mL ether aliquots. The final extraction step, however, was done by freezing the plasma layer in a dry ice–ethanol bath and decanting off the ether layer. Ether was evaporated from each test tube overnight in a fume hood

TABLE 2. Results of Exposure to Dietary Methlmercury in Fathead Minnows[a]

diet	mean ww (g)		mean total Hg in carcass ($\mu g \cdot g^{-1}$ ww)		mean GSI (%)		male T (ng mL^{-1})	female E2 (ng mL^{-1})	spawning success (%)
	male	female	male	female	male	female			
control	3.23 (0.28)	1.85 (0.07)	0.071 (0.001)	0.079 (0.002)	0.69 (0.36)	6.46 (0.53)	2.29 (0.60)	5.57 (1.27)	32 (10.2)
low	3.41 (0.22)	1.92 (0.15)	0.864 (0.038)	0.917 (0.027)	0.80 (0.06)	6.38 (0.60)	1.90 (0.33)	2.11 (0.52)	12 (4.9)
medium	3.64 (0.33)	1.79 (0.12)	3.557 (0.188)	3.842 (0.146)	0.74 (0.11)	3.82 (0.34)	1.06 (0.45)	1.08 (0.17)	0

[a] Numbers in parentheses represent 1 SE based on 5 tanks of fish per dietary treatment with 5 male and 5 female fish from each tank.

at 30 °C. Extracts were reconstituted in assay buffer for analysis. Testosterone and E2 calibration standards were also extracted (from solvent) and reconstituted in assay buffer with this procedure. Mean (± 1 SD) recovery of T and E2 from calibration standards was 91.4 (± 11.0)% and 97.4 (± 9.6)%. Samples were analyzed in duplicate and re-assayed if the coefficient of variation between duplicates exceeded 20%. Due to limited sample volumes, T was determined only for males, and E2 was determined only for females.

The accuracy of sex hormone assays was determined by triplicate analysis of a standard plasma sample from common carp (*Cyprinus carpio*) with each plate. Intra-assay coefficients of variation were 14.6% for T and 16.9% for E2. Inter-assay coefficients of variation were 23.6% for T and 26.6% for E2. Mean percent recoveries of triplicate spiked samples of carp plasma were 109.7% for T and 99.3% for E2. Method detection limits (*19*) of assays were estimated to be 0.028 ng mL^{-1} for T and 0.043 ng mL^{-1} for E2. Glassware used in assays was soap-washed and rinsed with reagent-grade water. Assay reagents and buffers were prepared with reagent-grade water.

Gonadal development of fathead minnows was used as an additional reproductive biomarker of MeHg exposure. Gonadal development was measured for each adult fish, regardless of whether it spawned, with the gonadosomatic index (GSI) expressed as the percentage of total body weight contributed by the gonads.

Statistical Analyses. For all variables except spawning success, repeated measures one-way analysis of variance (ANOVA) was performed to detect differences among treatments; each of the 15 exposure aquaria was considered as an experimental unit. Bonferroni post-hoc tests were used to determine differences between specific treatments. Differences in spawning success among treatments were detected with a χ^2 test. Ordinary least-squares regression models were used to assess relations between levels of sex hormones and gonadal development. Data were log-transformed, when appropriate, to meet the assumptions of parametric tests and were analyzed with SPSS for Windows software (version 10.1). In addition, quantile regression analysis (*27*) was used to estimate relations between concentrations of total mercury in fish carcasses and levels of sex hormones. Quantile regressions are appropriate for modeling the effects of limiting factors, such as toxicants, on biological responses (*28*), especially for relationships where heterogeneous responses result in "wedge-shaped" patterns of data that are heteroscedastic and violate assumptions of ordinary least-squares regression (*29*). Unlike ordinary least-squares regression, which estimates model functions through the center of data distributions (i.e., mean response), the upper regression quantiles (e.g., 90th) have slope estimates that are most consistent with the relation expected if mercury is actively limiting the maximum concentration of sex hormones in fish (*28*). The 75th, 80th, 85th, 90th, and 95th quantiles were estimated for the relation between total mercury in fish carcasses and concentration of T or E2 with the program BLOSSOM (*30*). The largest quantile for which the slope parameter was significant was used as a best estimate of the change in hormone concentration when mercury is the active limiting factor. A type I error (α) of 0.05 was used to judge the significance of all statistical tests.

Results and Discussion

Survival and Growth. Diets with elevated concentrations of MeHg were not lethal to fathead minnows. Mean survival of juvenile fathead minnows in 180-L aquaria ranged from 85% for fish fed the low-MeHg diet to 88% for fish fed the control and medium-MeHg diets. Most mortality was associated with deformities of unknown origin of the head or axial skeleton. Three adult male fish (one from each treatment) died of unknown causes while in the 50-L breeding aquaria.

Growth of fathead minnows was also unaffected by MeHg (Table 2). Mean wet weight (ww) and total length of test fish did not differ among treatments. Mean ww ranged from 3.23 (control) to 3.64 g (medium MeHg) in males (ANOVA, $F_{2,12} = 0.750$, $P = 0.49$) and from 1.79 (medium MeHg) to 1.92 g (low MeHg) in females (ANOVA, $F_{2,12} = 0.224$, $P = 0.80$). Mean total length of male fish did not vary among treatments, averaging 68 mm in control and low-MeHg diets and 69 mm in the medium-MeHg diet (ANOVA, $F_{2,12} = 0.104$, $P = 0.90$). Females from all dietary treatments averaged 59 mm total length (ANOVA, $F_{2,12} = 0.143$, $P = 0.87$).

Recent investigations of the effects of environmentally realistic concentrations of dietary MeHg on fish survival and growth, however, are equivocal. Methylmercury-contaminated diets reduced growth of male juvenile walleye but did not affect the survival of either sex (*31*). Likewise, Hammerschmidt et al. (*15*) observed no effect of diets artificially contaminated with MeHg on the survival of juvenile and adult fathead minnows; however, increased growth (i.e., hormesis) of male and female fathead minnows was reported. Furthermore, there was no effect of 0.5 $\mu g\ g^{-1}$ ww of dietary MeHg on weight of adult killifish, but male fish had increased mortality (*16*).

Dietary MeHg may affect survival and growth of fish as a result of behavioral alterations. Methylmercury is neurotoxic, yet its actions on the central nervous system of fish are poorly understood (*13*). Many studies with high concentrations of dietary MeHg (e.g., refs *32* and *33*) have documented suppression of appetite in fish followed by reduced growth and mortality. Hammerschmidt (*34*) noted increased vigor in fathead minnows exposed to low levels of dietary MeHg, possibly leading to the increased growth of fish. We observed no overt modifications of behavior of fathead minnows in this study, which may account for the lack of significant differences in survival and growth of fish among treatments.

Reproductive Biomarkers. Methylmercury suppressed levels of T in males and E2 in females (Table 2). Males fed the control diet had mean T concentrations that were 20% and 116% greater, respectively, than those fed the low- and medium-MeHg diets. Differences in T concentrations between males fed the control diet and those fed the medium-MeHg diet were statistically significant (ANOVA, $F_{2,12} = 4.941$, $P = 0.03$). Control females had mean E2 concentrations that were 164% and 416% greater than those fed the low- and medium-MeHg diets. Significant suppression of E2 levels occurred in females fed both low- and medium-MeHg diets

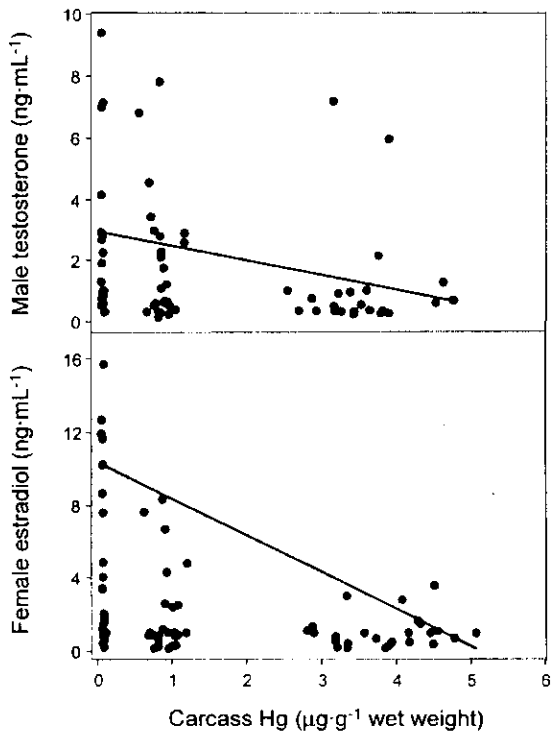

FIGURE 1. Relation between plasma testosterone and estradiol and concentration of mercury in the carcass of fathead minnows. Lines represent the 75th (males, top) and 90th (females, bottom) quantile.

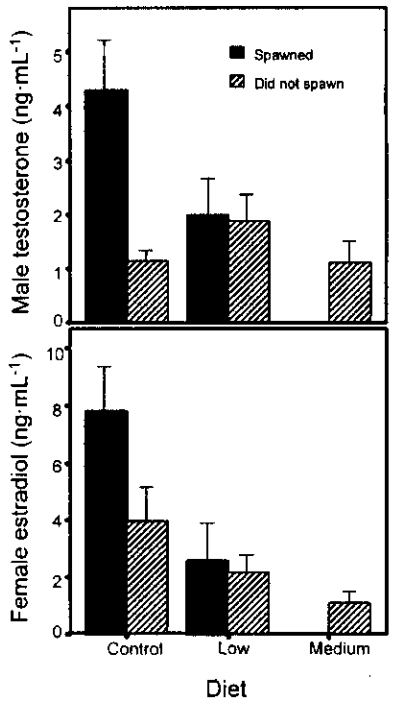

FIGURE 2. Mean plasma testosterone (males, top) and estradiol concentrations (females, bottom) of spawning and nonspawning fathead minnows fed diets with different levels of MeHg. Bars represent 1 SE.

(ANOVA, $F_{2,12} = 9.135$, $P < 0.01$). Moreover, maximum concentrations of T in males (75th quantile, $b_0 = 2.95$, $b_1 = -0.48$, $R = 0.05$, $P = 0.04$, $n = 69$) and E2 in females (90th quantile, $b_0 = 10.40$, $b_1 = -2.02$, $R = 0.65$, $P = 0.01$, $n = 62$) were inversely related to concentrations of total mercury in carcasses (Figure 1).

Differences in levels of sex hormones were also evident between spawning and nonspawning fish within dietary treatments (Figure 2). Spawning males fed the control diet had mean T concentrations 277% greater than male controls that did not spawn. However, the difference in T concentrations between spawning and nonspawning males fed the low-MeHg diet was only 5%. Female fish exhibited a similar trend. Spawning females fed the control diet had mean E2 concentrations 99% greater than female controls that did not spawn. Spawning females fed the low-MeHg diet had mean E2 concentrations 21% greater than those that did not spawn. The large difference in hormone levels between spawning and nonspawning controls was expected, given that increases in circulating hormone levels are critical for development and reproduction (*35*). Reproduction of control fish was initiated by elevated hormone levels (*36*). Those controls that did not spawn had much lower hormone levels, probably due to natural variation in steroid hormone synthesis and metabolism. The nominal differences observed in hormone levels between spawning and nonspawning fish fed the low-MeHg diet, however, indicate that elevation of T and E2 levels in male and female fish was limited by dietary MeHg.

Previous laboratory studies also have found that MeHg can suppress levels of sex hormones in fish. Plasma 11-ketotestosterone and E2 concentrations were suppressed in male and female tilapia (*Oreochromis niloticus*) dosed with capsules containing 0.1 or 1.0 mg of methylmercuric chloride (*14*). Exposure to high levels of MeHg in water inhibited steroidogenesis in male catfish (*Clarias batrachus*) (*37*). The ecological relevance of these findings are difficult to interpret, however, because test fish were not exposed to low levels of MeHg via the diet. This study, to our knowledge, is the first to report alterations in reproductive endocrine function in fish exposed to environmentally realistic concentrations of dietary MeHg.

The specific mechanism suppressing sex hormones in fish is unknown. Endocrine regulation of fish reproduction is mediated by the hypothalamic–pituitary–gonadal axis common to all vertebrate taxa. Hypothalamic secretion of gonadotropin-releasing hormone (GnRH) stimulates pituitary release of gonadotropins GTH-1 and GTH-2, which in turn act on the gonads to stimulate steroidogenesis (*38*). Disruption of single or multiple components of this axis could result in altered steroidogenesis (i.e., the observed suppression of T and E2). Exposure to high concentrations of waterborne MeHg can inhibit gonadotropic activity in the pituitary of catfish (*39*). Arnold (*14*) suggested that MeHg may suppress steroidogenesis by interfering directly with gonadal development. Future mechanistic studies could investigate effects of MeHg on molecular regulation of steroidogenesis, including expression and activity of steroidogenic enzymes (*40*).

Suppression of steroidogenesis can affect gametogenesis and the development of secondary sex characteristics, ultimately resulting in reduced reproductive success. Lower egg production by female fathead minnows fed MeHg-contaminated diets observed by Hammerschmidt et al. (*15*) was likely due to reduced levels of E2 and subsequently reduced vitellogenin, which is a substrate for developing eggs (*41*). Vitellogenin is produced in the liver of female fish and is induced by elevated levels of E2. Spermatogenesis was hindered in guppies (*Poecilia reticulata*) exposed to waterborne MeHg (*42*). Harries et al. (*43*) reported altered reproductive performance and abnormal steroid hormone profiles in fathead minnows exposed to 4-nonylphenol and related these alterations to inhibited development of secondary sex characteristics in males, including fewer nuptial

tubercles and a smaller dorsal fat pad. Moreover, a population of wild bream (*Abramis brama*) exposed to many contaminants in the Elbe River, Germany, was recently reported to have reciprocal relations between inhibitory effects on gonadal growth, levels of sex hormones, and expression of secondary sex characteristics (*3*). Further research could focus on the use of secondary sex characteristics as an indicator of altered reproduction and endocrine function of fish due to MeHg exposure.

Dietary MeHg inhibited gonadal development of female fathead minnows but not males (Table 2). The GSI of females fed the medium-MeHg diet was 40% less than that of females fed control or low-MeHg diets (ANOVA, $F_{2,12} = 8.686$, $P < 0.01$), which did not differ. The GSI of males was unaffected by dietary MeHg (ANOVA, $F_{2,12} = 0.406$, $P = 0.68$). Mean GSI in males ranged from 0.69% in controls to 0.80% in the low-MeHg treatment.

Studies examining effects of MeHg on gonadal development of fish have found mixed results. Hammerschmidt et al. (*15*) reported that dietary MeHg significantly reduced GSI in female but not in male fathead minnows, in agreement with our results. In contrast, dietary MeHg reduced GSI in male juvenile walleyes but not females (*31*). Very high concentrations of waterborne MeHg reduced the GSI of both male and female catfish (*37, 44*). However, GSI was not altered in male or female tilapia implanted with capsules containing methylmercuric chloride (*14*).

Histological examination of gonads may provide a more sensitive indicator than GSI for assessing effects of MeHg on gonadal development. Arnold (*14*), for example, reported atretic follicles and atrophied seminiferous tubules of female and male tilapia after treatment with MeHg even though GSI did not differ between treated and control fish. Juvenile male walleyes with reduced GSI also exhibited testicular atrophy, which likely contributed to the differences in GSI observed by Friedmann et al. (*31*).

Inhibition of gonadal development can affect reproductive potential of fish. Hammerschmidt et al. (*15*) reported that relative fecundity of female fathead minnows was negatively correlated with concentrations of total mercury in carcasses. This was likely due to altered steroidogenesis and gametogenesis of damaged reproductive tissues. Fathead minnows with small gonads in our study also had low levels of sex hormones (Figure 3). Testosterone in male fish ($r^2 = 0.14$, $P = 0.001$, $n = 69$) and E2 in female fish ($r^2 = 0.24$, $P < 0.001$, $n = 62$) were positively related to GSI. Females fed the medium-MeHg diet generally had the smallest gonads and lowest levels of E2, indicating that MeHg reduced the capacity of ovaries to produce sex hormones. McMaster et al. (*45*) reported that fish populations with reduced gonadal growth due to bleached kraft pulp mill effluent also exhibited reduced circulating levels of sex hormones due to reductions in capacity of gonadal tissues to synthesize steroids.

Biomarkers may be useful in assessing the reproductive health of wild fish populations exposed to MeHg, given that it is difficult to quantify reproductive success of fish in their natural environments. Measuring gonadal development, levels of sex hormones, and possibly vitellogenin could provide a method for monitoring subtle changes in reproductive performance of individuals that may adversely affect populations (*46*). This information could assist fishery managers in assessing the relative effects of MeHg on year-class strengths of wild populations of fish.

Reproductive Success. Dietary MeHg adversely affected reproduction of fathead minnows. The proportion of pairs of fish that spawned within 21 d after being placed into breeding aquaria decreased in a dose-dependent fashion ($\chi^2_{df=2} = 10.439$, $P < 0.01$; Table 2). Pairs fed the control diet accumulated little mercury (<0.1 $\mu g\ g^{-1}$ ww) and had a spawning success of 32% (i.e., eight of 25 pairs spawned).

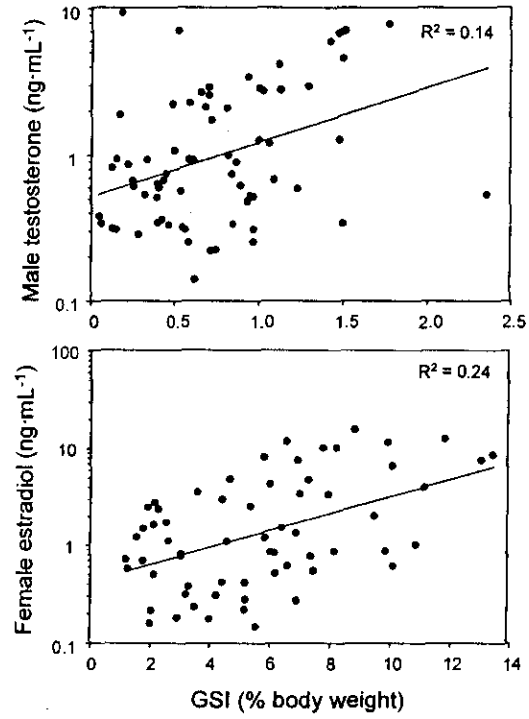

FIGURE 3. Relation between plasma testosterone (males, top) and estradiol (females, bottom) and the gonadosomatic index (GSI) of fathead minnows fed diets with different levels of MeHg.

Pairs fed the low-MeHg diet had mean total mercury concentrations slightly less than 1 $\mu g\ g^{-1}$ ww and a spawning success of 12%. No pairs in the medium-MeHg treatment spawned; their mean total mercury concentrations exceeded 3.5 $\mu g\ g^{-1}$ ww.

Dietary MeHg also delayed spawning of fathead minnows. Of those fish that spawned, pairs fed the low-MeHg diet required more days to spawn than those fed a control diet (ANOVA, $F_{1,5} = 7.992$, $P = 0.04$). Days to spawning averaged 12 and 17 d, respectively, for pairs fed control and low-MeHg diets.

Relative fecundity of female fathead minnows was not related to MeHg exposure. The number of eggs laid per gram of female fish was highly variable. Mean (± 1 SE) relative fecundity of females fed control and low-MeHg diets were 13.9 ± 7.3 and 35.5 ± 16.0 eggs g^{-1}, respectively.

Mercury concentrations that accumulated in carcasses of fathead minnows in this study are environmentally realistic. Piscivorous fish in low-pH lakes and newly flooded reservoirs, for example, often contain total mercury concentrations in axial muscle in the range of 0.5–3.0 $\mu g\ g^{-1}$ ww (*13*). Elevated concentrations can also occur in invertivorous fish, such as yellow perch (*Perca flavescens*; *47*). This suggests that wild fish populations could be at risk for impaired reproduction due to MeHg.

The adverse effects of MeHg on reproduction of fathead minnows were anticipated. Hammerschmidt et al. (*15*) also observed reduced spawning success and delayed spawning of fathead minnows fed MeHg-contaminated diets. However, they also observed reduced relative fecundity of female fish. We did not detect reduced relative fecundity presumably because sample sizes were small and egg production varied considerably within dietary treatments. Although not statistically significant, females fed the low-MeHg diet had higher relative fecundity than females fed the control diet. It is possible that females fed the low-MeHg diet had an increased relative fecundity (as compared to controls) because of

reduced egg size. The size of eggs are known to be affected by diet and other factors and can be related to egg quality (*41*). Egg size was not measured in this study, however.

Future work investigating effects of MeHg on reproduction of fish should also focus on early life stage and transgenerational effects. Fish embryos and larvae are exposed to MeHg in water and via maternal transfer (*23, 48*). Latif et al. (*49*) reported that environmentally relevant concentrations of waterborne MeHg reduced hatching success and lowered heart rates of walleye embryos. Altered reproduction, including reduced fertilization success and altered sex ratios, was noted in killifish exposed to maternally derived MeHg (*16*). Wiener and Spry (*13*) concluded that fish populations are most at risk from MeHg at existing exposure levels during embryonic and larval stages.

Ecological consequences of altered reproduction include population-, community-, and ecosystem-level effects. For example, atmospheric deposition of metals from a smelter near Flin Flon, MB, Canada, caused decreased recruitment in a population of white suckers (*Catostomus commersoni*) due to reduced spawning success of adults and reduced survival of eggs and larvae (*50*). Adams et al. (*2*) reported reduced fish community integrity (measured as species richness and relative abundance) in a reservoir subjected to many contaminants, including high concentrations of mercury, due to reproductive impairment of largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*). Ecosystem-level consequences of altered reproduction can occur if a fish population provides some service that is essential to ecosystem function. For example, alterations in reproductive performance of Pacific sockeye salmon (*Oncorhyncus nerka*) due to increased temperature can affect productivity of lake ecosystems where spawning occurs (*51*).

Concentrations of dietary MeHg regularly encountered by wild fish significantly suppressed levels of sex hormones, inhibited gonadal development, and reduced reproductive success of fathead minnows. This suggests that (i) suppressed hormone levels and inhibited gonadal development may be used to indicate altered reproduction of fish and (ii) reproduction of wild fishes may be adversely affected by MeHg. It is not known whether effects of MeHg on fish reproduction and endocrine function observed in the laboratory also occur in the field. Field studies indicate that alterations in reproductive biomarkers may occur in wild fish populations, yet causality is difficult to establish because of spatial and temporal variability and the presence of other contaminants (*2, 52, 53*). Further assessment of relations among exposure to MeHg, effects on biomarkers, and reproductive responses of wild fish populations is warranted.

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I, Faith Bugel, certify that on August 8, 2006, I filed the attached MICHAEL MURRAY REFERENCES IN SUPPORT OF TESTIMONY. An original and 9 copies were filed, on recycled paper, with the Illinois Pollution Control Board, James R. Thompson Center, 100 West Randolph, Suite 11-500, Chicago, IL 60601, and copies were served via United States Mail to those individuals on the included service list.

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