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Assessing the Ecological Risk of Mercury Exposure to Common Loons

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EXECUTIVE SUMMARY

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Title of Project: Assessing the Ecological Risk of Mercury Exposure to Common Loons

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Objectives of Wisconsin Loon Mercury Risk Assessment Project

Beginning in 1999, the Wisconsin Department of Natural Resources, U.S. Geological Survey, and the University of Wisconsin have collaborated on a research project designed to generate a scientifically defensible wildlife/mercury risk assessment. Primary project goals include: 1) determination of the toxicity risk posed to the Wisconsin common loon population from excess mercury exposure, and 2) development of a model that predicts changes in risk as a function of increasing or decreasing atmospheric mercury deposition rates and/or fish mercury concentrations.

The risk assessment focused on the common loon (*Gavia immer*), a species at risk to mercury exposure in Wisconsin. The project has been successful 1) quantifying the bioenergetic rates and pharmacokinetic rate constants necessary to produce a common loon mercury exposure model that predicts loon chick tissue Hg concentrations as a function of dietary Hg content and body mass and/or age (Fournier et al. 2002a, 2002b), 2) establishing a “critical life stage” mercury toxicity threshold (LOAEL - Lowest Observable Adverse Effect Level) by assessing the impacts of mercury exposure on loon chick growth, survival, behavior, and physiology (Kenow et al. 2003a), and 3) estimating the proportion of the Wisconsin common loon population at risk to mercury toxicity and predicting the population-level impacts (Meyer 2006). Research is ongoing to identify levels of mercury exposure associated with reduced common loon egg hatching rates. Model development continues to predict changes in loon mercury

exposure in response to changing atmospheric mercury concentrations.

With funding provided under the Wisconsin Focus on Energy (FOE) Grant, additional work was conducted to 1) finalize and validate predictions of the common loon Hg exposure model, 2) establish an accurate relation between mercury intake and blood mercury exposure, 3) collect tissue partitioning data, and 4) gather supplemental information concerning the effect of mercury exposure on the immune function and physiology of loon chicks.

To achieve these additional objectives, a chronic dosing study was conducted in 2003 using captive-reared loon chicks that were provided measured doses of methyl mercury in their diet. The information is being used to develop an empirical relation between prey mercury content and loon chick mercury exposure and refine our estimates of a LOAEL for mercury risk assessment. The results of this work will be used to establish the level of mercury in fish that safeguards survival and health of loon chicks reared on lakes in Wisconsin.

Summary of Results and Accomplishments

The first objective was to finalize and validate the common loon Hg exposure model. The model consists of a bioenergetics-based model used to predict food intake of common loon (*Gavia immer*) chicks as a function of body mass during development, and a pharmacokinetics model, based on first-order kinetics in a single compartment, used to predict blood mercury (Hg) level as a function of food intake rate, food Hg content, body mass, and Hg absorption and elimination. Predictions of the Hg exposure model were tested using captive loon chicks growing from hatch to 105 d that were fed rainbow trout (*Oncorhynchus mykiss*) with average concentrations of methylmercury of 0.02 (control), 0.4, and 1.2 $\mu\text{g g}^{-1}$ wet mass (delivered as CH_3HgCl); this allowed us to establish the relation between Hg intake and blood Hg exposure to fulfill Objective 2. Observed food intake matched predicted intake through age 50 d, but then predicted intake exceeded observed intake by an amount that grew progressively larger with age, reaching a significant overestimate of 28% by the end of the trial. Respiration in older, non-growing birds was probably overestimated by using field metabolic rates (as determined via doubly-labeled water, Fournier et al. 2002a) measured in younger, growing birds to parameterize the bioenergetic model. There was close agreement between simulations and measured blood Hg, which varied significantly with diet Hg and age. Although chicks may hatch with different blood Hg levels, as they age those differences become unimportant and beyond about 2 weeks their blood level is determined mainly by dietary Hg level. The model may also be useful for predicting Hg levels in adults, and in eggs that they lay, but the model's accuracy (in both chicks and adults) now needs to be tested in free-living birds.

Data collected under Objective 3 allows us to use the Loon Hg exposure model to predict loon chick tissue mercury concentrations as a function of age and mercury intake rates. We determined the distribution and accumulation of Hg in tissues of common loon chicks maintained for up to 15 weeks on either a control diet with no added CH_3HgCl or one containing either 0.4 or 1.2 $\mu\text{g Hg}$ as CH_3HgCl per g wet weight food. Total Hg and

methylmercury tissue concentrations were strongly positively correlated ($R^2 > 0.95$) with the amount of Hg delivered to individual chicks throughout the course of the experiment. The pattern of differential Hg concentration in internal tissues was consistent within each treatment: liver > kidney > muscle > carcass > brain. Feather Hg concentrations were consistently higher than that of internal tissues and represented an important route of Hg elimination. Feather mass accounted for 4.3 ± 0.1 (SE) percent of body mass yet $27.3 \pm 2.6\%$ of total Hg intake was excreted into feathers. Our calculations indicate that $26.7 \pm 4.9\%$ of ingested Hg was not accounted for, and thus either was never absorbed (e.g., Fournier et al. 2002b determined the oral bioavailability of MeHg delivered in this manner to be 83%) or was absorbed and subsequently eliminated in feces. With the additional excretion into feathers, 54% of ingested Hg was excreted. Evidence of mercury demethylation was found in the liver at all treatment levels (a smaller proportion of total Hg was in the form of MeHg compared to other tissues). This pattern was also present in the kidneys of chicks dosed at the 1.2- μg Hg/g level.

To meet the fourth objective, we conducted a dose-response laboratory study to quantify the level of exposure to dietary Hg that is associated with suppressed immune function in captive-reared common loon chicks. We used the phytohemagglutinin (PHA) skin test to assess T-lymphocyte function and the sheep red blood cell (SRBC) hemagglutination test to measure antibody-mediated immunity. The PHA stimulation index among dietary Hg treatment levels did not differ significantly from that of controls ($P = 0.15$). However, total antibody (IgM + IgG) production to the SRBC antigen in chicks treated with dietary MeHg was suppressed ($P = 0.04$) relative to chicks on control diets. A Least Squares Mean multiple comparison test indicated suppression of total-Ig production ($P = 0.025$ with comparison-wise alpha level = 0.017) between control and 0.4 μg Hg/g wet food intake treatment groups. We observed bursal lymphoid depletion in chicks receiving the 1.2 μg Hg/g treatment ($P = 0.017$) and a marginally significant effect ($P = 0.025$) in chicks on the 0.4 μg Hg/g diet. These findings suggest that common loon chick immune systems, particularly antibody production, may be compromised at an ecologically relevant dietary exposure level (0.4 μg Hg per g wet weight food intake).

The following manuscripts describing these findings have been prepared for publication in peer-reviewed outlets:

- Karasov W.H., Kenow K.P., Meyer M.W., Fournier F. 2007. A bioenergetic and pharmacokinetic model for exposure of common loon chicks to methylmercury. Submitted to *Environmental Toxicology and Chemistry* (in press).
- Kenow K.P., Meyer M.W., Hines R.K., Karasov W.H. 2007. Distribution and accumulation of mercury in tissues and organs of captive-reared common loon (*Gavia immer*) chicks. *Environmental Toxicology and Chemistry* (in press).
- Kenow K.P., Grasman K.A., Hines R.K., Meyer M.W., Gendron-Fitzpatrick A., Spalding M.G., Gray B.R. *Accepted with revision*. Effects of methylmercury exposure on the immune function of juvenile common loons. *Environmental Toxicology and Chemistry*.

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INTRODUCTION

Assessing the ecological risk of mercury (Hg) exposure to piscivorous wildlife is a priority issue for federal and state resource management agencies and industry (U. S. Environmental Protection Agency 1997a, 1997b). Regulators are interested in determining fish mercury concentrations that safeguard sensitive fish-eating wildlife populations. Fish have elevated mercury concentrations in many aquatic systems, a complex function of atmospheric deposition and methylation in acidic lakes and wetland complexes. However, the wildlife-Hg risk assessment model currently in use is compromised by a lack of relevant toxicological data from the laboratory and from the field, resulting in the use of numerous uncertainty factors (Meyer 1998). Depending on the assumptions used in the USEPA model, water column total mercury concentrations calculated to protect wildlife from adverse effects range from 400-4000 pg/L. This level of precision is inadequate for making regulatory decisions. To develop a scientifically defensible ecological risk assessment for Hg for wildlife, based on an at-risk species, we conducted a combination of laboratory and field studies of the common loon (*Gavia immer*).

The Wisconsin Department of Natural Resources (WDNR), U.S. Geological Survey (USGS), and the University of Wisconsin conducted a research project 1999-2000 designed to generate a scientifically defensible wildlife/mercury risk assessment model. Financial support for this project was provided by the Electric Power Research Institute, the Wisconsin Utilities Association, the Wisconsin Department of Natural Resources, and the U.S. Geological Survey. The project was successful in 1) quantifying bioenergetic rates and pharmacokinetic rate constants necessary to produce a bioenergetic model that predicts loon chick tissue Hg concentrations as a function of dietary Hg content and body mass and/or age (Fournier et al. 2002a) and 2) in assessing the impacts of mercury exposure on loon chick growth, survival, behavior, and physiology (Kenow et al. 2003a; Kenow et al. Unpubl. data). However, due to problems with dose delivery and purity problems with parent material used in the second year of the study, we were unable to establish an accurate relation between mercury intake, blood mercury exposure, and tissue partitioning. This information is needed to finalize and validate a bioenergetics-based toxicokinetic model. The toxicokinetic model predicts common loon blood Hg concentrations as a function of the mercury content of fish they consume. In addition, our toxicology results were inconclusive with respect to whether the current mercury exposure levels in Wisconsin negatively impact loon chick health. We observed no evidence of mercury toxicosis or negative effects on survival, behavior, or chick physiology at ecologically relevant dose levels. However, we did detect suppression ($P = 0.068$) of T lymphocyte-mediated immunity with increased dietary mercury, at exposure levels commensurate with that found in chicks from low pH lakes in northern Wisconsin. The Focus on Energy grant provided an opportunity to conduct additional dose-response work with a focus on immune function to increase the power of these analyses.

Therefore, the goal of this study was to collect the additional information needed to complete the development of the loon/mercury risk assessment model. Specific objectives of this project were to:

- 1) establish an accurate relation between dietary mercury intake and blood mercury

- exposure via a 105-day exposure study,
- 2) finalize and validate predictions of a bioenergetics-based Hg exposure model,
 - 3) model the partitioning of MeHg among chick tissues, and
 - 4) gather supplemental information concerning the effect of mercury exposure on the immune function and physiology of loon chicks.

METHODS

Source and Husbandry of Common Loon Chicks

The study was conducted in summer 2003 at the USGS Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI. Common loon eggs were collected from a four-county region of northern Wisconsin as authorized in U.S. Fish and Wildlife Service Scientific Collecting Permit MB030466-0 and WDNR Permit SCP-WCR-15-D-03. We collected eggs at lakes where loons are known to have elevated levels of Hg exposure (from low [< 6.3] pH lakes) and at lakes where loons are known to have low levels of Hg exposure (from neutral [> 6.3] pH lakes). Other details concerning the source, collection, and incubation of eggs are available in Kenow et al. (2003a). Twenty-three hatched chicks were marked with a unique four-digit web tag and randomly assigned to one of three dietary Hg treatment groups. Two died over the course of the trial because of bacterial infection. Husbandry of chicks was described previously (Kenow et al. 2003a). Handling and care of chicks were approved by the Animal Care and Use Committee of the UMESC and comply with the Animal Welfare Act. Loon chicks were fed rainbow trout *ad libitum*. The rainbow trout were reared at the UMESC by the Center fish culturist. Daily food intake was documented for each chick throughout the experiment. The mass (± 0.1 g) of each fish provided to each chick was determined. Each loon chick was weighed daily (± 0.1 g for chicks < 1000 g, ± 1 g for chicks > 1000 g) prior to being fed each morning.

Delivery of Dietary MeHg

Stock dose solutions were prepared by dissolving CH_3HgCl (COA indicated 98.5% purity; Crescent Chemical Co., Inc., Islandia, NY, USA; independent analysis [R. Rossmann, EPA Great Lakes Lab, Grosse Ile, MI] verified the purity at 96.5%) in reagent-grade acetone. New solutions were prepared every 2 to 4 weeks. The preparation and stability of dosing solutions were carefully monitored throughout the experiment to ensure that concentrations did not change over time. Daily Hg doses were administered via a rainbow trout (*Oncorhynchus mykiss*) containing a gelatin capsule (Fournier et al. 2002), with the prescribed daily dose of CH_3HgCl depending upon food intake so that each loon within an experimental block received either a diet with no extra Hg or 0.4 or 1.2 μg Hg per g wet weight fish. The experiment was terminated when the chicks were 105 d old. Technicians, biologists, and consulting veterinarian assigned to the study were blinded from the treatment levels of the chicks to ensure care, treatment, monitoring, measurement, conduct of assays, and tissue collection was conducted without bias.

Blood Residue Analysis and Hematology

Blood samples were collected weekly from each chick by venipuncture of the jugular vein and stored in a cryotube for subsequent Hg analysis, although only biweekly samples were analyzed. The Hg content of whole-blood samples was determined by cold-vapor atomic absorption spectrophotometry (detection limit = 0.01 µg Hg/ml; EnChem, Inc., Madison, WI, USA) using standard methods (USEPA 1984; AOAC 1990).

We obtained differential white blood cell and red blood cell counts from blood samples drawn from the jugular vein at 14, 35, 56, 77, and 98 days of age. In addition, standardized dry slide blood chemistry analyses were conducted using a Kodak 700XR (Ortho Diagnostics, Rochester, New York) and protein electrophoresis was used to quantify plasma protein fractions (Cray and Tatum 1998). Analysis was conducted at the Avian and Wildlife Laboratory, University of Miami Comparative Pathology, (Miami, Florida) for analysis

Bioenergetic and Pharmacokinetic Model for Exposure of Common Loon Chicks to Methylmercury

Bioenergetic rates and pharmacokinetic rate constants necessary to produce a bioenergetic model that predicts loon chick tissue Hg concentrations as a function of dietary Hg content and body mass and/or age were quantified during field and laboratory studies that were conducted in 1999-2000 (Fournier et al. 2002a; Fournier et al. 2002b). Rates or rate constants measured include (i) daily Hg dose (the product of the loon's feeding rate and dietary Hg concentration (Fournier et al. 2002a), (ii) bioavailability, or how much of a dose appears in the blood or body (Fournier et al. 2002b), and (iii) elimination rate constants which can be used to determine the proportion that remains just before the next daily dose (Fournier et al. 2002b). Using these basic building blocks, we constructed a model to predict whole-body and blood Hg concentrations for free-living common loon chicks as a function of fish Hg content and developmental stage (pre- and post-molt), which influence both intake and elimination rates. The situation of wild loons, which consume Hg in fish daily but require more than a day to eliminate it (Fournier et al. 2002b), was modeled applying the pharmacokinetics of multiple dosing (Ritschel 1999). Model development and validation reported here compliment loon dose-response laboratory studies that have been reported elsewhere (Kenow et al. 2003a). Predictions were tested using captive loon chicks on prescribed diets containing varying levels of Hg.

Daily food intake (I , units g wet mass d⁻¹) was modeled using a balanced energy equation (Weathers 1996):

$$I = (R + P)/(MEC \cdot e_{food} \cdot w_{food}) \quad (1)$$

where R is respiration (kJ d⁻¹), P is production (kJ d⁻¹), MEC is the coefficient of metabolizability of the food energy (unitless), e_{food} is the food's gross energy content (measured to be 26.9 kJ g⁻¹ dry mass in rainbow trout (*Salmo gairdneri*) fed to loons; Fournier et al. 2007) and w_{food} is the dry:wet ratio of the food (measured to be 0.3 g dry mass g⁻¹ wet mass; Fournier et al. 2007). The respiration term includes energy expended

for basal metabolism, thermoregulation, physical activity, the heat increment of feeding, and any excess respiration associated with formation of new tissue. The *MEC* term incorporates energy losses in feces and urine. Production, mainly related to growth, was calculated as the product of daily mass change ($g, g \text{ wet mass d}^{-1}$) and the energy density of the bird ($e_{bird}, kJ g^{-1} \text{ wet mass}$).

Blood Hg content on any given day (C_t , units $\mu g g^{-1} \text{ wet blood}$) was modeled using an open, single-compartment model (Ritschel 1999):

$$C_t = (I \cdot c_{food} \cdot \alpha) / V + (C_{t-1} \cdot e^{-k}) \quad (2)$$

where I is daily food intake, c_{food} is the food's Hg content (units $\mu g g^{-1} \text{ wet mass}$), α is the oral bioavailability (i.e., proportion absorbed) of dietary Hg, V is the effective distribution volume of Hg in the body (units $g \text{ wet blood}$), C_{t-1} is the blood Hg content on the day before, and e^{-k} is the persistence factor, a factor which accounts for the decline of blood Hg due to elimination and growth dilution (units d^{-1}) since the previous dose the day before.

Tissue Sampling

The chicks were sacrificed at the age of 105 days. Brain, liver, kidney, and pectoralis muscle were removed and representative samples of each designated for residue analysis and stored at $-70^\circ C$. Additional tissues, including the thymus, thyroids, lung section, spleen, gonads, heart, bursa, bone sample, and pancreas, were removed for histopathological examination and represented about 3% of the total body mass. The remaining carcass was stored at $-70^\circ C$ and later thawed for feather removal. The carcass was homogenized after feather removal and consisted of non-pectoralis muscle, bone, skin, fat, gastrointestinal tract, remnants of tissues not removed for histopathological samples, and other miscellaneous tissues.

Feathers were plucked from nine body regions (head/neck, dorsal body, ventral body, apertium, tail, legs, primaries, secondaries, and remaining wing feathers including all coverts, scapulars and tertials), cleaned, and stored separately to prevent cross-contamination among regions. Feather samples were dried at about $20^\circ C$ and weighed before homogenization to get total mass of each body region. A representative subsample of feathers from each body region was digested and analyzed for total Hg. The Hg content of feather samples (with the exception of secondaries) was determined by cold-vapor atomic absorption spectrophotometry (detection limit = $0.01 \mu g \text{ Hg/g}$; EnChem, Inc., Madison, WI, USA) using standard methods [USEPA 1984; AOAC 1990]. Method blanks and standard reference material were processed and analyzed concurrently with samples.

Blood (subset of available samples), carcass homogenate, and tissue samples (including secondary feathers) were digested and analyzed for total Hg by tin chloride reduction, dual gold amalgamation, and cold-vapor atomic fluorescence spectrometry (CVAFS) detection (detection limit = 0.05 ng/g ; Frontier SOP FGS-069; Frontier Geosciences Inc., Seattle, WA, USA). Methylmercury was analyzed by aqueous phase ethylation, isothermal gas chromatography separation, and CVAFS detection (detection limit = 1.2

ng/g; Frontier SOP FGS-070; Frontier Geosciences Inc., Seattle, WA, USA). Reported values are instrument blank and preparation blank corrected but not adjusted for percent recovery.

Assays for Immunocompetence

The phytohemagglutinin (PHA) skin response test was used to measure T lymphocyte-mediated immunity following the protocol of Grasman et al. (1996) using a 0.1 ml dose of 1 mg/ml PHA-P (Sigma, St. Louis, Missouri) in sterile phosphate buffer saline (PBS). The test was conducted in conjunction with the hemagglutination test described below in 99-day-old chicks. One wing web was randomly selected to receive an injection of the PHA-P solution, while the other received a 0.1 ml injection of PBS only. The thickness of each wing web was measured (± 0.01 mm) using a pressure-sensitive caliper (Model No. 304-196, Dyer Co., Lancaster, Pennsylvania) prior to and 24 ± 1 hr following injection. A stimulation index (SI) was calculated as the change in the thickness of the PHA-inoculated wing web minus the change in the thickness of the PBS-injected wing.

The sheep red blood cell (SRBC) hemagglutination test was used to measure antibody-mediated immunity. Common loon chicks were immunized via the jugular vein with a 1% suspension of SRBC in normal saline at 99 days of age. Plasma samples were collected 6 days later (105 days of age), snap frozen in liquid nitrogen, and stored at about -70° C until shipped for processing. Antibody activities were measured using a modification of the microtiter procedure of Gross and Siegel (1980, 1981).

Histological Examinations

We also assessed indicators of immunocompetence through histological examinations of the thymus, bursa, and spleen collected from loon chicks. Upon removal, spleen mass (SPLEEN-MA) was determined to the nearest 0.001 g and dimensions (length and width) of thymus was determined to the nearest mm. SPLEEN-MA and thymus area (THYMUS-AR = length*width) were standardized to body mass. Qualitative and quantitative measures of lymphoid depletion in these organs were obtained. Lymphoid depletion in the thymus was indexed as the average number of Hassel's corpuscles (THYMUS-HC) observed in 4 fields of thymus sections (determined at 200x), an evaluation of lymphoid depletion over the entire slide (THYMUS-LD) was scored, and a subjective evaluation of the amount of thymic tissue present on the slide (THYMUS-TS) was scored as abundant or scarce. Observations of the spleen included the number of germinal centers around arterioles (SPLEEN-RE; determined at 200x) and level of lymphoid depletion over the entire section (SPLEEN-LD; 40x and 100x) ranked. Bursa sections were evaluated for lymphoid depletion over the entire sections at 40x and 100x (BURSA-LD). Bursal wall thickness (BURSA-WT) and number of full medullary and cortical areas observed in a 200x field (BURSA-FL) provided indices of involution.

Statistical Analyses

Methylmercury exposure model. For analyses, the data for each common loon chick were randomly assigned to either a calibration data set or to a validation data set. We developed a linear summary and predictive model for log(respiration) as a function of log(body mass) in free-living and captive common loon chicks using analysis of covariance (Wilkinson 1992). Blood Hg concentrations as a function of sampling time

and dose group were analyzed by analysis of variance. When comparing observed with predicted blood Hg concentrations in figures, we plotted box plots that were notched at the median and the upper and lower maximum extent which correspond to the 95% confidence intervals on the median. If the intervals around two medians do not overlap, one can be confident at about the 95% level that the two population medians are different (Wilkinson 1992). We also approximated the variance of predicted values (I and C) based on the measured variances of the parameters (Table 1), using procedures for estimating variance of sums, products and quotients (Mood et al. 1974).

Distribution of tissue mercury. Outcomes were analyzed using linear models and residual maximum likelihood (SAS® linear mixed modeling, proc mixed; SAS 2003). Variances were allowed to vary by treatment. We used Tukey-Kramer-adjusted Least Squares Mean [LSM] multiple comparison tests to examine outcome differences among dietary Hg treatment levels. A bivariate normal model with random loon effects was used to test for differences in slopes of log-transformed liver- and kidney-total Hg and MeHg concentrations with amount of dietary Hg delivered. For body burden calculations, we assumed blood volume to be 10% of juvenile loon body mass (Sturkie 1976) and carcass mass was adjusted to account for tissues removed for histological examination. Mercury excretion in feces was determined as the difference in Hg delivered by means of dose delivery from resulting Hg body burden.

Immune function. Continuous outcomes (SI, total antibody, IgG, THYMUS-AR, THYMUS-HC, BURSA-WT, BURSA-FL, SPLEEN-MA, and SPLEEN-RE) were analyzed using linear models and residual maximum likelihood while the balance of the outcomes comprising those of ordered categorical data only (THYMUS-TS, THYMUS-LD, SPLEEN-TS, SPLEEN-LD, BURSA-LD) were analyzed using proportional odds models (Allison 1999). Proportional odds models modeled the probability of response levels with higher scores. Linear and proportional odds models were fit using SAS® linear mixed modeling and logistic regression procedures, respectively (proc mixed and proc logistic; SAS 2003). For the larger datasets (i.e., SI and antibody), variances were allowed to vary by year or treatment when evidence for such heterogeneity was indicated by visual inspection of residuals and by the small-sample variant of the Akaike Information Criterion (AICc). Where variances were presumed heterogeneous, degrees of freedom were estimated after Kenward and Roger (1997).

SI and SRBC antibody estimates were adjusted for the effects of gender, year and the potential confounders of the occurrence of aspergillosis and bacterial infection. Similar adjustment was not made for the histological outcomes because those outcomes were associated with sample sizes inadequate (i.e., $n = 21$) to support moderately complex models. Visual inspection, however, indicated no evidence of concern for the effects of gender, aspergillosis, or bacterial infections. Due to the perceived interest in no and lowest observed effect levels, treatment results are presented graphically (means with standard errors) and as differences from reference levels. Confidence intervals for differences among continuous and categorical responses were t- and profile likelihood, respectively (Allison 1999).

RESULTS AND DISCUSSION

Methylmercury exposure model

The Hg exposure model that we provide predicts the blood Hg concentration as a function of time in common loon chicks based on the blood Hg concentration at hatch, the body mass as a function of time post-hatch, and the diet Hg content. Observed food intake based on the bioenergetics-based model matched predicted intake of captive-reared loon chicks through age 50 d, but then exceeded observed intake by an amount that grew progressively larger with age, reaching a significant overestimate of 28% by the end of the trial. Respiration is the largest component of the loon chick's energy budget (Weathers 1996). We used pooled field metabolic rate data as measured with doubly labeled water from Fournier et al. 2002a and Kenow et al. 2003b (Figure 1) to estimate respiration as a function of body mass in the bioenergetics module. Respiration was probably overestimated in older, non-growing birds by using field metabolic rates measured in younger, growing birds. Based on mass changes of the nine validation chicks (Figure 2A) and the allometric relations for respiration (Figure 1) and tissue energy content, we estimated daily respiration, production, and total metabolizable energy (their sum) (Figure 2B). Food intake was then estimated taking into account the metabolizable energy content of fish and compared with daily measures of food consumed by the common loon chicks *ad libitum* (Figure 2C). Observed intake matched the predicted intake best at the youngest ages and both peaked around age 50 d, when observed intake began to decline. Predicted intake, which we estimate has a CV of about 0.24, exceeded observed intake by an amount that grew progressively larger with age. After week 12 (84 d), the predicted intake was outside (above) the 95% confidence interval of the observed intake, which had a CV that ranged 0.19–0.26. By the end of the trial, predicted intake overestimated observed intake by 28% (Figure 2C). It is possible that the accuracy of our predictive model could be improved by measuring the field metabolic rate of older, larger common loon chicks and loon adults.

Applying the pharmacokinetics module, there was close agreement between simulations and measured blood Hg, which varied significantly with diet Hg and age. At hatch, blood Hg concentration was significantly higher in chicks from eggs collected at high Hg lakes ($2.03 \pm 0.52 \mu\text{g Hg g}^{-1}$ wet blood, $n = 10$) than from eggs collected at low Hg lakes (1.49 ± 0.55 , $n = 11$) ($F_{1,19} = 5.2$, $P = 0.034$). Although chicks may hatch with different blood Hg levels, as they age those differences become unimportant and beyond about 2 weeks their blood level is determined mainly by dietary Hg level.

Chick blood-Hg levels sampled every other week (Figure 3) differed significantly according to treatment group ($F_{2,97} = 57$, $P < 0.001$) and time ($F_{8,97} = 7.9$, $P < 0.001$). Figure 3 includes data from the calibration chicks, and these data were used as a guide during model development. For example, the first version of the model lacked a factor for growth dilution, but it became apparent that the decline in blood Hg of control and lower dose birds could not be adequately explained without it. As a second example, the fit between weeks 9 and 11 was best when we assumed that k_e declined exponentially with age rather than linearly. Once the model was developed using these data, we independently cross checked it by comparing its predictions with data from nine other

chicks (those represented in Figure 2A). Data from those nine chicks were not inspected until the model had been finalized.

We predicted blood levels of the nine validation chicks based on their body masses (daily means for each dose group in Figure 4A) and measured daily doses of Hg (units $\mu\text{g d}^{-1}$; daily means in Figure 4B). In this comparison, we did not use predicted Hg inputs based on the bioenergetics module, because we wanted to evaluate the performance of the pharmacokinetic model independently of the performance of the bioenergetics model. The predicted blood Hg contents as a function of age were then compared with the values measured in the loons every other week (Figures 5A, 5B). The starting point for all predictions was the mean blood Hg of chicks at hatch, $1.74 \pm 0.25 \mu\text{g g}^{-1}$ wet blood ($n = 9$). There was variability in the predictions, plotted as 95% confidence intervals in Figure 5, because of the variation in daily dose ($\text{CV} = 0.24$; Figure 4), but we expect that is somewhat an underestimate. The approximate $\text{CV} = 0.38$ for the predictions, when accounting also for variance in other measured parameters such as distribution space and rate constants (Table 1). The model predicted the transition from generally steady, lower values in each dose group prior to about age 50 d to a continuous rapid increase in blood Hg with time after age 50 d. Also, the model predicted fairly well the differences in blood concentration between the dose groups at the older ages. Although the model predicted the rapid decline in blood Hg of undosed controls during the first few weeks after hatch, it overestimated by as much as 143% the blood levels during those weeks in the no-dose controls (best seen in Figure 5B) and by as much as 68% in the other dose groups. These are statistically significant overestimates, based on our approximate $\text{CV} = 0.38$ and assuming $n = 9$ loons. Based on the apparent overestimate of blood Hg in the very young chicks (Figure 5), it appears that either the k_e is higher than we assumed, or that the rate constant for growth dilution was underestimated. Two solutions to this problem are to derive the rate constants with nonlinear curve fitting procedures by fitting our data to the model, or to measure directly the elimination rate constant in younger loon chicks. However, we think that resolving this is not necessarily a high priority. Whatever the exact kinetics of the young chicks, their blood Hg concentrations later in life are primarily determined by diet Hg content and the slow kinetics of elimination that follow the completion of feather growth (Fournier et al. 2002b).

We also predicted the blood levels based solely on body masses of the other 12 calibration chicks, and the effective diet Hg contents of dosed birds, which were 0.4 or 1.2 $\mu\text{g Hg g}^{-1}$ wet fish. At 105 d post-hatch, the model's predictions based on this information were, respectively, 3.2 and 9.4 $\mu\text{g Hg g}^{-1}$ wet blood. These model estimates are 8-13% higher than the respective estimates plotted in Figure 5 (2.8 and 8.7), because they are influenced as well by the predictions of the bioenergetics model that overestimated food intake. Thus, these predictions, which are based just on body mass changes and diet Hg content, were about 30% above the mean blood Hg concentrations of the nine individuals in the validation group at age 105 d (Figure 5). Considering that predictions have an approximate $\text{CV} = 0.38$, the model seems fairly accurate at the older ages of loons.

Accumulation of Hg in tissues

Tissues of 15 chicks sacrificed at 105 days old ($n = 5$ per dietary Hg treatment) and three chicks sacrificed at 35 days old (all three chicks on the 0.4- μg Hg/g diet) were included in the analyses. The Hg exposure resulting from our experiment (Figure 3) brackets the ecologically relevant exposure levels for juvenile common loons (Evers et al. 1998). Tissue Hg concentrations in chicks at 105 days old varied significantly (LSM, $P < 0.007$) among dietary Hg treatment levels (Table 2). Both total Hg and methylmercury tissue concentrations were strongly positively correlated ($R^2 > 0.95$) with the amount of Hg delivered to individual chicks throughout the course of the experiment. The pattern of differential Hg concentration in internal tissues was consistent within each treatment: liver > kidney > muscle > carcass > brain.

Feather Hg concentrations were consistently higher than that of internal tissues and represented an important route of Hg elimination. Feather mass accounted for 4.3 ± 0.1 (SE) percent of body mass yet $27.3 \pm 2.6\%$ of total Hg intake was excreted into feathers (Table 3). Feathers contain a greater proportion of dietary Hg during or shortly after cessation of feather growth (e.g., see comparison of tissue Hg levels between 35- and 105-day-old chicks on the 0.4- μg /g diet in Table 2). Our estimate of Hg elimination by means of feathers does not take into account dietary Hg excreted with loss of the second down. Therefore, our reported excretion rates to feathers are probably underestimated and excretion by means of feces overestimated. The proportion of Hg deposited as percent of body burden or percent of dietary intake did not vary significantly (LSM, $P > 0.07$) with treatment in any of the tissues. The tissue partitioning coefficients for muscle, brain, and organs was approximately 10x for the 0.4 dose and 30-40x for the 1.2 dose, while the feather coefficient was 20x for the 0.4 dose and 60-80x for the 1.2 dose.

Among 35- and 105-day-old chicks, Hg residue in tissues was largely MeHg (generally >90%). However at 105 days, 70% (95% CL = 0.63, 0.77) of liver total Hg was in the form of MeHg across treatment levels, and MeHg comprised about 73% of Hg in kidneys of the 1.2- μg /g treatment group. This finding is probably the result of a demethylating enzyme system. Such systems are thought to occur primarily in the liver and kidney of birds.

Immune function

Data collected in 2003 supplemented findings from our previous work with loon chicks reared in captivity during 1999 and 2000. The PHA stimulation index among dietary Hg treatment levels did not differ significantly from that of controls ($P = 0.15$; Figure 6). We found that common loon chicks had an overall lower level of SI response (i.e., control mean = 0.23 mm) compared to other species (reference means range from 0.6 to 1.2 mm; [Grasman et al. 1996; Grasman and Scanlon 1995]). Despite the lack of statistically significant treatment effects in the SI response, we are struck by the 40% lower SI for the 0.4 μg Hg/g treatment group, along with more subtle decreases in the other treatment groups. Generally a 30-50% decrease in response from baseline indicates biologically significant immunosuppression (Grasman et al. 1996); a 50-60% decrease is the maximum observed in several studies where T cells have been eliminated by radiation or immunosuppressive drugs.

However, total antibody (IgM + IgG) production to the SRBC antigen in chicks treated with dietary MeHg was suppressed ($P = 0.04$) relative to chicks on control diets (Figure 7). A Least Squares Mean multiple comparison test indicated suppression of total-Ig production ($P = 0.025$ with comparison-wise alpha level = 0.017) between control and 0.4 $\mu\text{g Hg/g}$ wet food intake treatment groups. We observed bursal lymphoid depletion in chicks receiving the 1.2 $\mu\text{g Hg/g}$ treatment ($P = 0.017$) and a marginally significant effect ($P = 0.025$) in chicks on the 0.4 $\mu\text{g Hg/g}$ diet. These findings suggest that common loon chick immune systems may be compromised at an ecologically relevant dietary exposure level (0.4 $\mu\text{g Hg}$ per g wet weight food intake). The mean level of total Ig was depressed 58% in the 0.4 $\mu\text{g Hg/g}$ treatment group, a level that is likely biologically significant. Further, we feel that the effect of low level (i.e., 0.08 $\mu\text{g Hg/g}$) exposure warrants further testing as the results suggest suppression of titer in the 0.08 $\mu\text{g Hg/g}$ treatment (mean appear to be 54% that of titer levels in controls) as well (Figure 7). The apparent lack of effect on antibody-mediated immunity at the 1.2 $\mu\text{g Hg/g}$ dose level may be the result of a lack of analytical power. Alternatively, these data may represent a true paradoxical effect where toxicity increases at low dose but decreases at higher dose levels. Statistical power in our analysis suffers as a result of limited sample sizes among treatment groups, the consequence of a limited supply of common loon chicks available for study. To enhance analytical power, follow-up studies should focus on low-level Hg effects.

We found evidence to suggest that dietary MeHg contributed to lymphoid depletion in the bursa. Bursal lymphoid depletion may provide a mechanistic explanation for the observed reduction in antibody production to the SRBC antigen. B-lymphocytes mature in the bursa and are responsible for secreting antibodies. Hence, there is a logical connection between reduced numbers of developing B-cells and a suppressed antibody response of the mature B-cells. However, this doesn't rule out the possibility of other mechanisms acting directly on mature B-cells. We detected an effect of lake source on lymphoid depletion in bursa tissue samples as well. Chicks originating from low-pH lakes were more likely to have higher levels of lymphoid depletion compared to chicks from neutral-pH lakes ($P < 0.05$). There was also evidence of an increased heterophil:lymphocyte ratio in chicks from low pH lakes (ANOVA, $F = 4.78$, $P = 0.03$) (as well as in the 1.2 $\mu\text{g Hg/g}$ treatment group), which may be indicative of stress in this cohort (as noted previously, blood Hg concentration at hatch was significantly higher in chicks from eggs collected at low pH lakes).

CONCLUSIONS

To understand and predict blood Hg concentrations in common loons, we sought a model based primarily on principles in energetics and pharmacokinetics. There was close agreement between simulations and measured blood Hg, which varied significantly with diet Hg and age. Although chicks may hatch with different blood Hg levels, as they age those differences become unimportant and beyond about 2 weeks their blood level is determined mainly by dietary Hg level. The model may also be useful for predicting Hg

levels in adults, and in eggs that they lay, but its accuracy in both chicks and adults needs to be tested in free-living birds. This model can also be coupled with tissue partitioning coefficients to predict 105 day old loon chick organ mercury concentrations as a function mercury intake rates.

Interpretation of tissue Hg residue data allows us to establish a relation between Hg intake and the amount and proportion of Hg deposited in various tissues, provides a means of estimating Hg contamination in the environment more quantitatively than has been done previously using common loon chick tissue residue information, describes the relations among loon tissue Hg concentrations, and assesses the extent of demethylation in juvenile common loons. The information may be used to provide perspective and a useful means of interpreting the variety of measures of Hg exposure reported in the literature.

The results of this study suggest that common loon chick immune systems may be compromised at an ecologically relevant dietary exposure level (0.4 μg Hg per g wet weight food intake). Lack of more convincing effects of dietary MeHg in juvenile common loons at ecologically-relevant dietary mercury levels (i.e., 0.4 μg Hg/g) is likely the result of protection afforded by rapid MeHg elimination rates during feather development (Fournier et al., 2002b). We also observed no negative effects on growth or survival (Kenow et al., 2003a) and marginal behavioral effects (Kenow et al., Unpublished results) on common loon chicks and concluded that the high rate of MeHg excretion measured in our study provided a large measure of protection from MeHg toxicity. These results should not be extrapolated beyond the 105-day trial period, because blood mercury levels were on an upward trajectory and tissue mercury levels may increase considerably. Further research is needed on the risk that MeHg exposure poses to the immune system, including consequences on increased susceptibility to infections and general fitness, individual survival, and potential implications on population dynamics.

We observed differences in several response variables with respect to source of eggs. Earlier we reported growth effects related to chick lake source (Kenow et al. 2003). Behavioral differences between loon chicks from low pH and neutral pH lakes were also detected (Kenow et al. Unpubl. data) suggesting that chicks from low pH lakes were generally less motivated or more lethargic. Chicks from low-pH lakes exhibited higher blood mercury levels at hatch compared to chicks from neutral-pH lakes, and the lake source effects we observed may have been related to *in ovo* exposure to MeHg. Alternatively, other factors related to lake pH, such as altered availability of toxic metals (Wiener 1987; Scheuhammer 1991) or essential elements (Scheuhammer 1991), or there may be some difference in overall fitness of parents nesting on low-pH vs. neutral-pH lakes and have persistent consequences on chick fitness. Common loon egg injection experiments are currently being conducted in Wisconsin to isolate the impact of *in ovo* MeHg exposure on egg hatching success and loon chick fitness.

Evidence of reduced immune function in chicks receiving ecologically relevant doses (0.4 μg Hg/g diet wet weight) indicate that the preliminary estimate (subject to peer

review) of the loon chick Lowest Observable Adverse Effect Level (LOAEL) = 0.4 ug Hg/g wet weight fish. The preliminary estimate of the No Observable Adverse Effect Level (NOAEL) for loon chicks = 0.08 ug Hg/g wet weight fish. Typical prey items consumed by loon chicks in Wisconsin include yellow perch (*Perca flavescens*), bluegill (*Lepomis macrochirus*), and Cyprinidae sp., up to 16mm length (Merrill et al. 2005, WDNR Unpubl. data). Samples of these prey items collected within our study area in 2001 (n=50 lakes) range 0.01 – 0.40 ug Hg/g wet weight fish (WDNR unpubl. data), with highest fish Hg concentrations found on acidic lakes.

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TABLES

Table 1. Parameters used in the bioenergetic and pharmacokinetic models, and their typical variances.

Symbol	Parameter and its units	Value ¹	CV ²	Source
<i>I</i>	Daily food intake, g wet mass d ⁻¹	30 – 350	0.24	This study
<i>R</i>	Respiration, kJ d ⁻¹	600 - 2500	0.15	Fournier et al. 2002a Kenow et al. 2003b
<i>P</i>	Production, kJ d ⁻¹	0 – 750	0.15 ³	Kenow et al. 2003a
<i>MEC</i>	Coefficient of metabolizability, unitless	0.64 – 0.83	0.18	Fournier et al. 2007
<i>e_{food}</i>	Food gross energy, kJ g ⁻¹ dry mass	26.9	0.03	Fournier et al. 2007
<i>w_{food}</i>	Food dry:wet ratio, unitless	0.3	0.05	Fournier et al. 2007
<i>C</i>	Blood Hg content, µg g ⁻¹ wet blood	0.02 – 9	0.3	This study
<i>c_{food}</i>	Food Hg content, µg g ⁻¹ wet food	0.02 – 1.2	0.05	This study
<i>α</i>	Oral bioavailability of dietary Hg	0.83	0.07	Fournier et al. 2002b
<i>V</i>	Distribution volume of Hg in the body, proportion of body mass	0.43	0.19	Fournier et al. 2002b
<i>k</i>	Rate constant for elimination (and growth dilution) of Hg, d ⁻¹ g ^{0.25}	-0.0447 to -1.411	0.25	Fournier et al. 2002b

¹value shown is either mean or typical range (if value changes with age or some other factor), ²CV = standard deviation/mean; typical value shown, ³based on variation among individuals in mass gain per day when growth rate is maximal

Table 2. Mercury (Hg) concentrations ($\mu\text{g/g}$ wet weight [fresh weight in feathers]; mean \pm SE) in various captive-reared common loon organs and tissues in relation to dietary mercury exposure ($\mu\text{g Hg/g}$ wet weight food intake delivered as methylmercury chloride) at 35 and 105 days old. Levels of Hg in controls reflect low-level background Hg in forage fish provided to the chicks.

Organ/tissue	Percent solids	35 days at 0.4 $\mu\text{g/g}$ $n = 3$	105 days at control $n = 5$	105 days at 0.4 $\mu\text{g/g}$ $n = 5$	105 days at 1.2 $\mu\text{g/g}$ $n = 5$
Brain	32.3 \pm 0.3	0.43 \pm 0.02	0.09 \pm 0.02	0.88 \pm 0.06	3.10 \pm 0.13
Kidney	27.0 \pm 1.1	0.65 \pm 0.01	0.20 \pm 0.07	2.29 \pm 0.15	8.13 \pm 0.52
Liver	28.2 \pm 0.4	0.99 \pm 0.02	0.42 \pm 0.11	4.03 \pm 0.28	14.86 \pm 2.12
Pectoralis muscle	31.9 \pm 1.2	0.46 \pm 0.05	0.10 \pm 0.02	1.23 \pm 0.07	4.50 \pm 0.25
Blood	17.5	0.58 \pm 0.10	0.18 \pm 0.05	1.98 \pm 0.34	7.67 \pm 0.90
Carcass	47.6 \pm 1.1	0.44 \pm 0.03	0.10 \pm 0.03	1.11 \pm 0.05	4.01 \pm 0.24
Feather region					
Apertium	94.7 \pm 0.1	20.17 \pm 0.59	1.36 \pm 0.40	26.92 \pm 4.11	85.98 \pm 3.74
Head/neck	94.5 \pm 0.2	19.00 \pm 1.18	1.33 \pm 0.41	25.46 \pm 3.67	81.60 \pm 3.57
Leg	94.5 \pm 0.1	19.80 \pm 0.38	1.26 \pm 0.39	25.36 \pm 4.33	82.92 \pm 5.39
Dorsal body	94.5 \pm 0.1	18.00 \pm 0.80	1.26 \pm 0.39	21.86 \pm 2.24	72.06 \pm 4.59
Wing	92.5 \pm 0.7	18.13 \pm 0.90	1.06 \pm 0.23	23.30 \pm 3.56	65.26 \pm 10.79
Tail	94.3 \pm 0.1	18.43 \pm 0.58	1.08 \pm 0.34	21.16 \pm 2.82	63.40 \pm 5.43
Ventral body	87.9 \pm 1.4	18.53 \pm 1.07	1.14 \pm 0.26	20.26 \pm 2.14	62.52 \pm 6.91
Primaries	93.8 \pm 0.2	16.60 \pm 0.23	0.98 \pm 0.29	19.30 \pm 2.77	59.68 \pm 8.57
Secondaries	94.5 \pm 0.3	15.47 \pm 0.77	1.10 \pm 0.23	16.70 \pm 1.31	58.66 \pm 1.08

Table 3. Amount of mercury (Hg) (mean \pm SE) in organs and tissues of 105 day old juvenile common loons expressed as a percentage of the total body burden of Hg and as a percentage of dietary Hg intake (includes background Hg level in forage fish). Dietary Hg was delivered as methylmercury chloride.

Tissue	Wet mass of tissues at 105 d (g)	Percent of body burden			Percent of dietary intake		
		Control <i>n</i> = 5	0.4 $\mu\text{g/g}$ <i>N</i> = 5	1.2 $\mu\text{g/g}$ <i>n</i> = 5	Control <i>N</i> = 5	0.4 $\mu\text{g/g}$ <i>n</i> = 5	1.2 $\mu\text{g/g}$ <i>n</i> = 5
Brain	9.5 \pm 0.1	0.2 \pm 0.02	0.1 \pm 0.01	0.1 \pm 0.00	0.2 \pm 0.04	0.1 \pm 0.00	0.1 \pm 0.00
Kidney	23.9 \pm 1.1	0.9 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.2	0.5 \pm 0.03	0.6 \pm 0.02
Liver	59.3 \pm 3.9	4.9 \pm 0.9	3.5 \pm 0.3	3.7 \pm 0.2	4.0 \pm 1.0	2.2 \pm 0.2	2.7 \pm 0.2
Pectoralis muscle	483.9 \pm 18.0	9.6 \pm 0.7	9.0 \pm 0.3	9.4 \pm 0.5	7.8 \pm 1.3	5.8 \pm 0.3	6.8 \pm 0.4
Blood	328.2 \pm 13.6	10.9 \pm 1.0	9.4 \pm 1.5	11.0 \pm 1.4	9.0 \pm 1.9	6.2 \pm 1.1	8.0 \pm 1.1
Carcass	2,187.6 \pm 98.0	39.6 \pm 3.8	35.6 \pm 2.4	37.9 \pm 0.0	33.1 \pm 7.0	22.6 \pm 1.1	27.6 \pm 2.2
Feathers:	138.9 \pm	34.0 \pm 5.8	41.7 \pm 2.6	37.2 \pm 3.3	27.9 \pm 7.5	27.2 \pm 3.3	26.8 \pm 1.9
Leg	5.1	0.7 \pm 0.1	1.0 \pm 0.1	1.0 \pm 0.1	0.6 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1
Apertium	2.8 \pm 0.2						
Secondary	3.2 \pm 0.3	0.9 \pm 0.4	1.1 \pm 0.2	1.3 \pm 0.2	0.8 \pm 0.4	0.8 \pm 0.2	0.9 \pm 0.1
Tail	5.3 \pm 0.2	1.2 \pm 0.2	1.3 \pm 0.1	1.3 \pm 0.1	1.0 \pm 0.2	0.8 \pm 0.1	1.0 \pm 0.1
Dorsal body	5.0 \pm 0.3	1.1 \pm 0.2	1.6 \pm 0.2	1.3 \pm 0.1	0.9 \pm 0.3	1.0 \pm 0.2	0.9 \pm 0.1
Head/neck	6.0 \pm 0.4	1.6 \pm 0.4	2.0 \pm 0.3	1.6 \pm 0.1	1.4 \pm 0.5	1.3 \pm 0.3	1.2 \pm 0.1
Primary	6.7 \pm 0.3	1.9 \pm 0.4	2.3 \pm 0.1	2.4 \pm 0.1	1.6 \pm 0.5	1.5 \pm 0.1	1.8 \pm 0.1
Wing	11.5 \pm 0.3	2.3 \pm 0.5	3.1 \pm 0.3	3.0 \pm 0.4	1.9 \pm 0.5	2.0 \pm 0.3	2.1 \pm 0.2
Ventral body	35.5 \pm 1.2	8.1 \pm 1.1	11.6 \pm 1.0	9.6 \pm 1.5	6.6 \pm 1.5	7.6 \pm 1.1	6.9 \pm 1.1
	63.0 \pm 2.7	16.2 \pm 2.8	17.9 \pm 1.0	15.8 \pm 1.6	13.2 \pm 3.5	11.6 \pm 1.1	11.3 \pm 0.8

FIGURES & LEGENDS

Figure 1. Respiration of common loon chicks, measured with doubly labeled water. Although separate linear regressions are shown for the free-living loons (dashed line) and for captives (solid line), the two groups did not differ significantly by analysis of covariance. Based on least squares linear regression of all the log-transformed values, the allometric relation is $\text{kJ/d} = 9.82 (+ 2.46) \text{ mass}_g^{0.67 (+ 0.08)}$, $r = 0.85$. Each point represents a measure on an individual.

Figure 2. (A) Average body mass (+ SEM) during development of the nine validation chicks. (B) Predicted growth energy (Production), respiration, and their sum (Metabolizable energy) in common loon chicks, based on the body mass pattern in shown in panel A. The lines are LOESS fits to the daily estimates. (C) Predicted food intake, based on panel B, compared with observed *ad libitum* food intake of the nine validation chicks. The solid line is the LOESS fit to the daily estimate of food intake. The unfilled circles with bars are means (+ SEM) of food intake calculated each day through age 35 d, and weekly thereafter.

Figure 3. Blood mercury concentrations of the calibration loon chicks by dose group (lower dose equivalent to $0.4 \mu\text{g g}^{-1}$ wet food consumed; higher dose $1.2 \mu\text{g g}^{-1}$ wet food consumed). Values shown are means + SEM ($n = 4$ for each group).

Figure 4. Body mass (A) and daily mercury dose (B) of the nine validation chicks in the three dose groups (higher dose, $n = 4$; lower dose, $n = 3$; no-dose controls, $n = 2$). There were no discernable differences in body mass among the dose groups, but the daily doses of mercury (Hg) differed considerably by experimental design. The Hg was delivered as methylmercury orally in a capsule inside a fish. The Hg dose of the no dose controls represents the Hg intake due to background level in the fish and in the capsule. The lines in each panel were fit using a LOWESS smoother.

Figure 5. Predicted and observed blood mercury content of the nine validation chicks plotted on an arithmetic scale (A) and \log_{10} scale (B). In each panel, the dense lighter grey bars represent the 95% confidence intervals on the median predicted daily blood mercury (Hg), based on the daily doses and body masses shown in Figure 5. The upper values correspond to the high dose, the middle values to the low dose, and the lowest values to the no dose controls. The darker symbols are box plots of blood Hg, which was measured biweekly in individuals of each of the three dose groups. The box plots are cleanly separated according to dose group in both panels at age 20 d and beyond. Each plot's full extent represents the 95% confidence intervals on the median of measured blood Hg within each of the three dose groups (higher dose, $n = 4$; lower dose, $n = 3$; no-dose controls, $n = 2$). If the intervals around two medians do not overlap, one can be confident at about the 95% level that the two population medians are different.

Figure 6. Mean skin response (± 1 SE) to PHA injection in juvenile common loons with respect to treatment levels of dietary Hg ($\mu\text{g Hg/g}$ wet food intake as CH_3HgCl) and lake source pH category. Means have been adjusted for treatment, lake source, sex, year, and evidence of infection.

Figure 7. Mean primary antibody response (\pm SE) 5 d after SRBC immunization in juvenile common loons with respect to treatment levels of dietary Hg ($\mu\text{g Hg/g}$ wet food intake as CH_3HgCl) and lake source pH category. Means have been adjusted for treatment, lake source, sex, year, and evidence of infection.

Figure 1

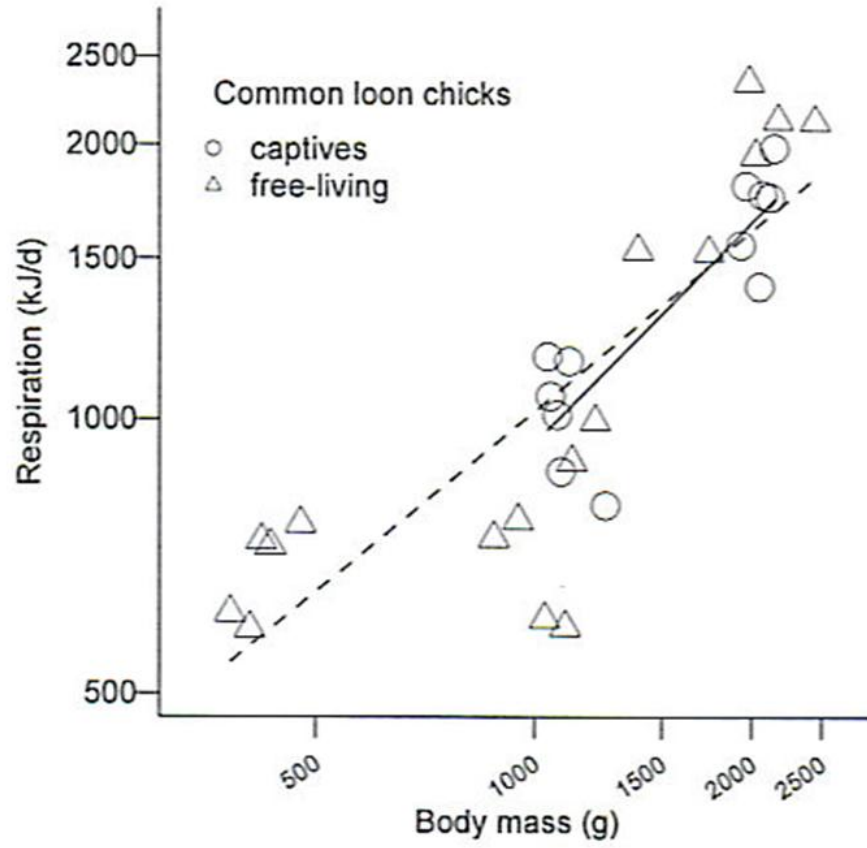


Figure 2

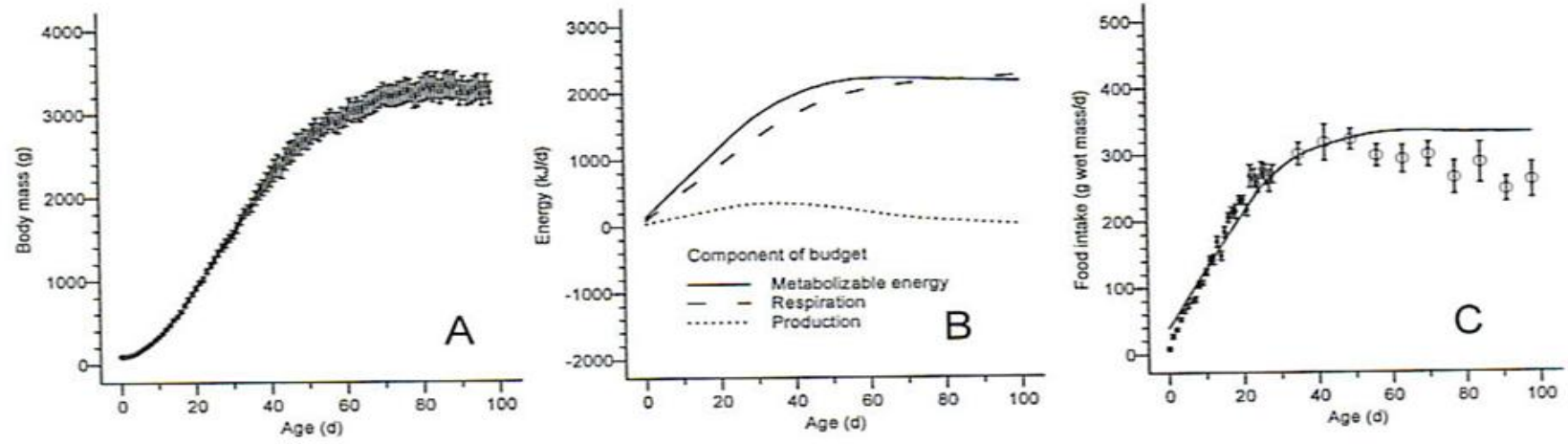


Figure 3

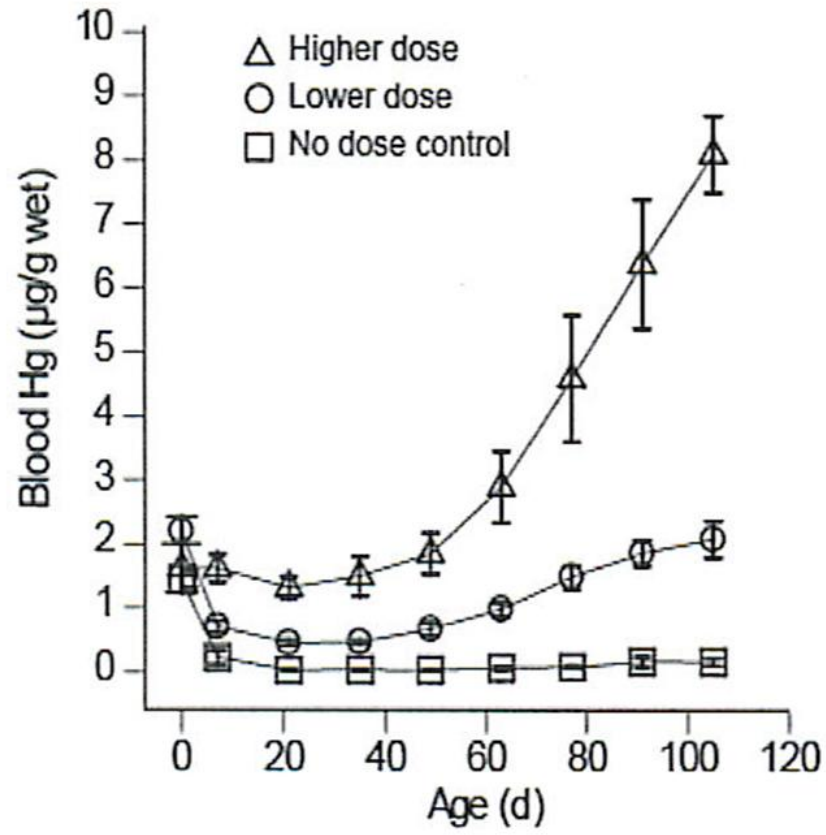


Figure 4

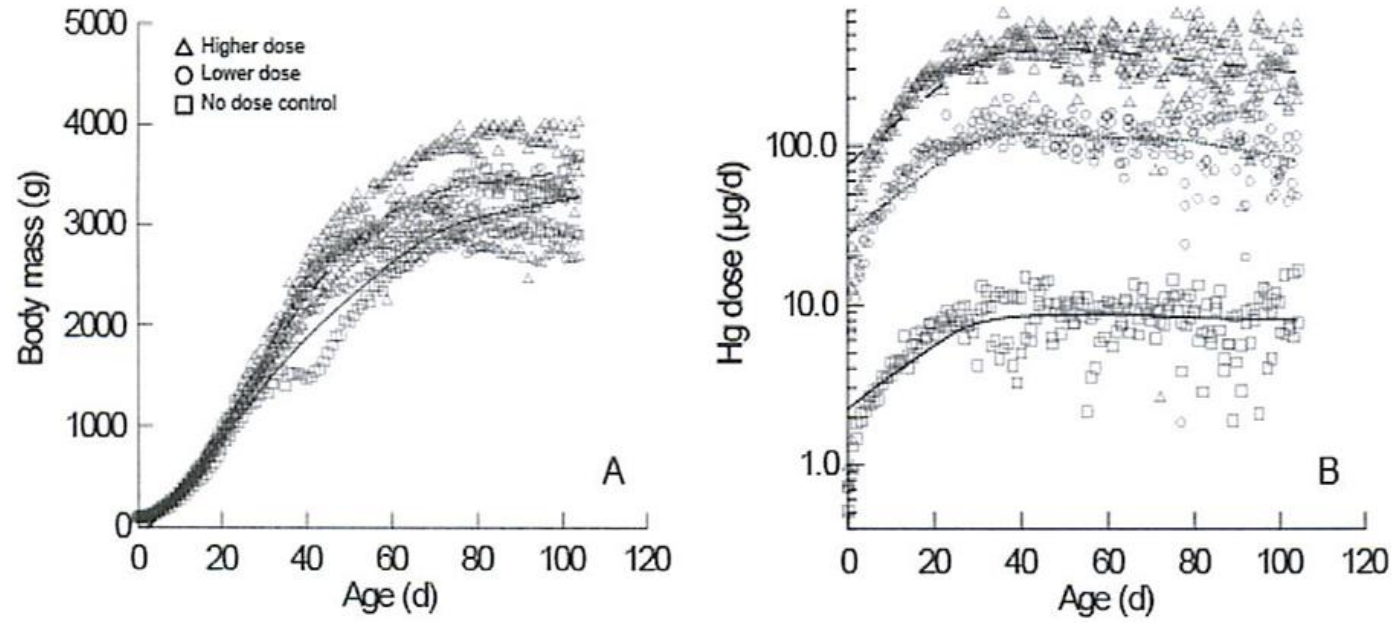


Figure 5

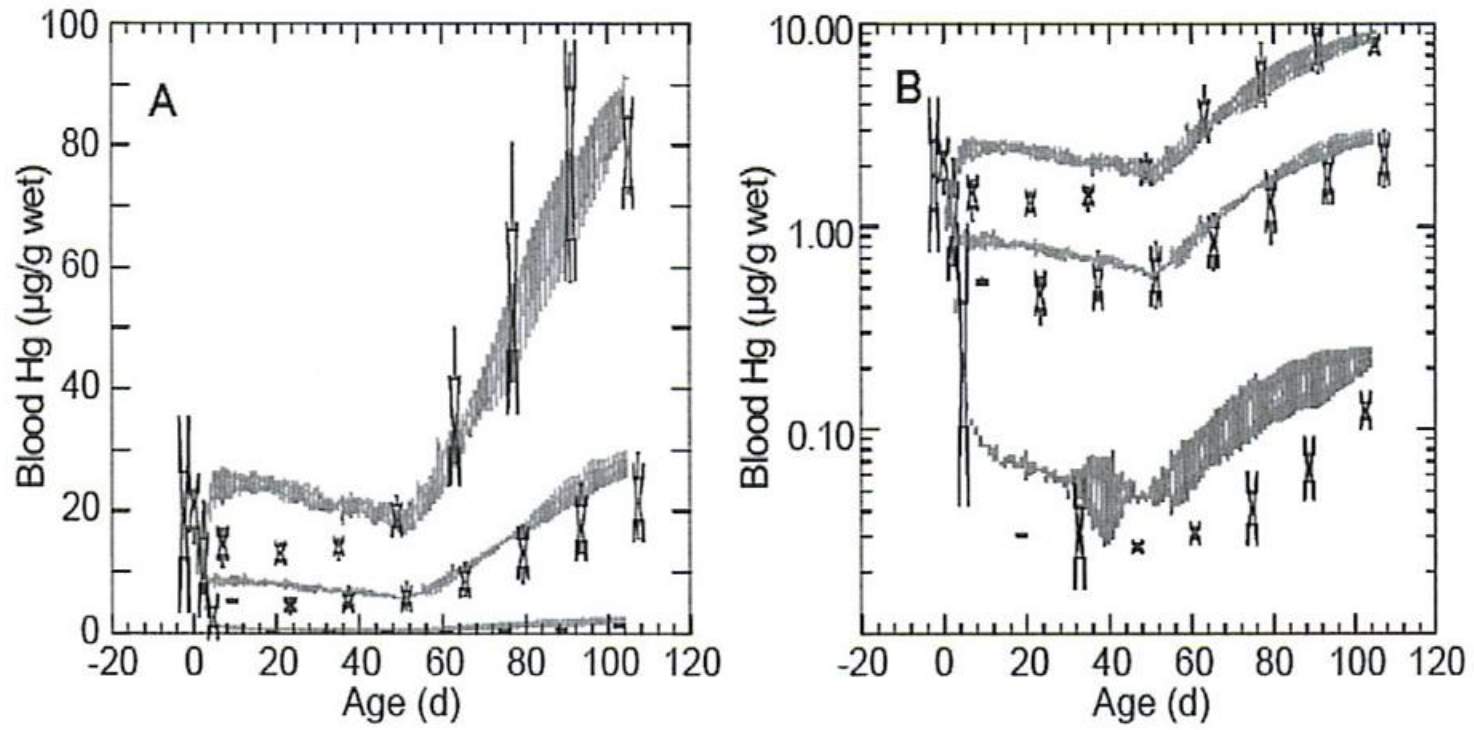


Figure 6

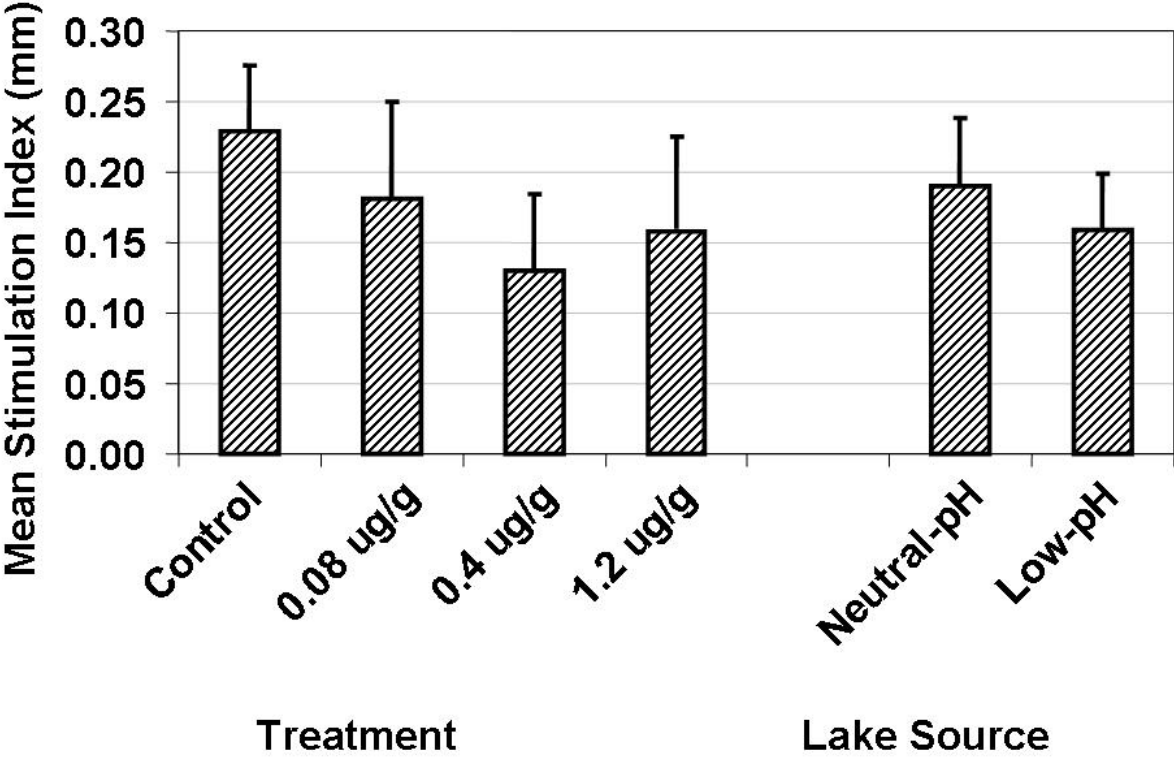


Figure 7

