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The oral bioavailability and toxicokinetics of methylmercury in common loon (*Gavia immer*) chicks[☆]

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Abstract

We compared the toxicokinetics of methylmercury in captive common loon chicks during two time intervals to assess the impact of feather growth on the kinetics of mercury. We also determined the oral bioavailability of methylmercury during these trials to test for age-related changes. The blood concentration-time curves for individuals dosed during feather development (initiated 35 days post hatch) were best described by a one-compartment toxicokinetic model with an elimination half-life of 3 days. The data for birds dosed following completion of feather growth (84 days post hatch) were best fitted by a two-compartment elimination model that includes an initial rapid distribution phase with a half-life of 0.9 days, followed by a slow elimination phase with a half-life of 116 days. We determined the oral bioavailability of methylmercury during the first dosing interval by comparing the ratios of the area under the blood concentration-time curves ($AUC_{0 \rightarrow \infty}$) for orally and intravenously dosed chicks. The oral bioavailability of methylmercury during the first dosing period was 0.83. We also determined bioavailability during both dosing periods using a second measure because of irregularities with intravenous results in the second period. This second bioavailability measure estimated the percentage of the dose that was deposited in the blood volume (f), and the results show that there was no difference in bioavailability among dosing periods. The results of this study highlight the importance of feather growth on the toxicokinetics of methylmercury.

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1. Introduction

Contamination of aquatic ecosystems stemming from anthropogenic sources of mercury continues to increase (Swain et al. 1992; Monteiro and Furness, 1997). It is therefore conceivable that the concentration of mercury in many aquatic organisms will also continue to increase. The primary chemical form of mercury found in aquatic organisms is the toxic organometal methylmercury (MeHg) (Thompson and Furness, 1989; Bloom,

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1992; Kim et al., 1996). High levels of MeHg in aquatic organisms, especially fish, represent both an ecological and human health concern because the ingestion of contaminated fish is the primary input of mercury to humans and piscivorous wildlife. Assessing the ecological risk of mercury exposure to piscivorous wildlife is a priority issue for government resource management agencies and industry because regulators are interested in determining fish mercury concentrations that safeguard sensitive piscivorous wildlife populations. However, the development of a risk assessment model based on current knowledge of the effects of MeHg on birds 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; Fimreite, 1974).

Wildlife species at the top of aquatic food chains, such as piscivorous birds, are particularly susceptible to the negative effects of mercury because of the biomagnification of MeHg in these food chains (Morel et al., 1998). Also, because there is a negative relationship between lake pH and alkalinity and the mercury content of piscivorous fish (Cope et al., 1990), birds feeding on prey from acidic lakes may be exposed to higher levels of mercury than those feeding on neutral-pH lakes. We have focused our research effort on the common loon (*Gavia immer*) for two reasons. First, correlative studies have demonstrated that common loons are sensitive to the toxic effects of mercury (Barr, 1986; Scheuhammer and Blancher, 1994; Nocera and Taylor, 1998). Reproduction is the most sensitive endpoint of MeHg toxicity in birds. Altered adult behavior and egg laying, embryo mortality (thus reduced hatching rates), increased chick mortality and altered chick behavior have all been documented as the result of mercury exposure in birds (Heinz, 1979; Thompson, 1996; Nocera and Taylor, 1998). Second, common loons are at risk of the greatest mercury exposure in many aquatic system as they are high trophic level, long-lived, obligate piscivores. Loons that frequently breed on low-pH, low-alkalinity lakes, and therefore feed on fish containing higher levels of mercury, had lower hatching success than birds breeding on neutral-pH lakes that consume less contaminated prey (Meyer et al., 1995, 1998).

A first step in understanding the accumulation of mercury in common loons is to determine the fate of ingested mercury. The amount of MeHg in the body not only depends on the concentration in

food, but also on the physiological mechanisms that regulate the uptake, distribution, and excretion of this compound in the organism. Birds accumulate mercury from their food and they eliminate it through feces, feathers and eggs. Therefore, the body burden of mercury in birds is a balance between rates of intake and excretion. Because MeHg has a high affinity for the sulfhydryl groups found in the keratin of feathers, much of the mercury that piscivorous birds take up from their food goes into growing plumage. Once feathers are fully-grown they become physiologically isolated from the rest of the body (Stettenheim, 2000), and therefore prevent further influx of mercury from the body to the feathers. Mercury levels in the body might therefore be relatively low during feather growth and increase once feathers are fully-grown. Feathers represent an important excretory route for mercury in birds, and several studies have documented changes in body burden before and after molting in adult birds (Furness et al., 1986; Braune and Gaskin, 1987a,b).

We conducted a study of the toxicokinetics of MeHg in common loon chicks during their development to determine the effect of feather growth on oral bioavailability and rate of elimination of MeHg. We used a pharmacological approach to determine bioavailability, a method that has not been used extensively in ecotoxicology (McCloskey et al., 1998). The approach entails calculating the ratios of the area under the blood mercury vs. time curves of orally and intravenously dosed individuals (Welling, 1986). To assess the impact of feather growth on the mercury kinetics we dosed the birds at times in their development that corresponded to a period of high feather growth and a period following completion of feather development.

2. Material and methods

2.1. Animals and husbandry

Loon eggs were collected in northern Wisconsin and transported to the Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI, where they were hatched in incubators at 37.5 °C and a relative humidity of 65–70%. Each chick was marked with a numbered webtag to aid in identification. Chicks were held indoors in 0.7×2.9 m raceways (4 chicks per raceway)

containing approximately 25 cm of water, resting platform, and brooder light until they were approximately 30-days-old. Room lighting was maintained at a 16L:8D light cycle. Chicks were then transferred to 48-m² outdoor ponds flooded to a depth of 0.6 m and equipped with resting platform and brooder light. A constant supply of well water (12° C) was supplied to indoor raceways and outdoor ponds.

Chicks were fed rainbow trout (*Oncorhynchus mykiss*) that had been reared at the UMESC by the Center fish culturist. The diet was supplemented with thiamine and multi-vitamins. A sample of fish provided to the loons was assessed for mercury content.

2.2. Dose preparation and administration

Eight loon chicks were used in the bioavailability trials. Four birds were randomly assigned to either the oral or the intravenous (IV) dose group and dosed 2 times to examine the possible effect of feather development on the pharmacokinetics of mercury. These two sampling times corresponded to when feather development was at its peak (35 days old) and when feathers were fully grown (84 days old).

The oral dosing solution was prepared by dissolving methyl mercuric chloride (99% purity, ICN Biochemicals, Aurora, OH, USA) in acetone to obtain a target concentration of 10 mg of methyl mercuric chloride per ml of solution. The concentration of the oral dosing solution was determined prior to its use, and it did not differ from the target concentration.

A single pulse oral dose was administered during and after feather development. Dosing solution was dispensed into a size 00 gelatin capsule (Torpac Inc., Fairfield, NJ, USA) to obtain a target dose of 500 µg of MeHg per kg body mass. The open capsules containing the solution were then placed in a fume hood to permit the acetone to evaporate. Once the acetone had evaporated the capsules were capped. Each dosing capsule was placed inside a freshly killed rainbow trout which was immediately fed to the birds. Birds were then observed to ensure fish were not regurgitated—none were during the experiment. We determined the accuracy of our gelcap procedure for delivering the appropriate amount of methylmercury by placing a target amount of solution in gelcaps and having the latter analyzed for mercury content.

The concentration of mercury in the gelcaps did not differ from the target concentration.

Blood samples (1 ml) were collected from all orally dosed birds during the feather development period before the administration of the dose ($t=0$) and subsequently at 0.5, 1, 4, 7, 14, 21, 28 and 49 days post administration, for a total of 9 blood samples from each bird.

During the second part of the bioavailability trials (post feather development period), blood samples were collected from the orally dosed birds before administration ($t=0$) and sequentially at 0.5, 1, 4, 7, 14, 21, 28 and 35 days post administration, and weekly thereafter until approximately 121 days post administration for a mean of 19 blood samples from each bird.

Intravenous dosing was accomplished by injecting a solution containing a target dose of 200 µg methyl mercuric chloride per kg body mass into the jugular vein of anesthetized loon chicks using a precision Hamilton glass syringe. The position of the needle in the vein was confirmed before and after injection by aspirating blood into the syringe. The injection solution was prepared by first dissolving 1 g of methyl mercuric chloride in 10 ml of acetone resulting in a concentrated stock solution of 100 mg methyl mercuric chloride per ml acetone. We then transferred a 1-ml aliquot of the concentrated stock solution into a 100-ml volumetric flask and added saline solution (0.9% NaCl) to a final volume of 100 ml. The latter solution was stirred for 48 h to ensure complete solubility (Eisler, 1987). The final target concentration of the intravenous dosing solution was 1000 mg methyl mercuric chloride per ml of solution. The concentration of the intravenous dosing solution was determined prior to its use, and it did not differ from the target concentration.

During the feather development period, a 1-ml blood sample was collected from each bird in the intravenous dose group before administration ($t=0$) and subsequently at 0.5, 1, 4, 7, 14, 21, 28, 35 and 49 days post administration for a total of 10 blood samples per bird.

Blood collection from the intravenously dosed chicks during the post feather growth period consisted in drawing a 1-ml blood sample before administration ($t=0$) and sequentially at 0.04, 0.125, 0.25, 1, 4, 7, 14, 21 and 35 days post administration, and weekly thereafter until approximately day 90 post administration, for a mean of 17 blood samples per bird.

The Hg content (total mercury) of all of our samples (fish, gelpcaps, dosing solutions, and whole blood) was determined by cold-vapor atomic absorption spectrophotometry (detection limit = $0.01 \mu\text{g Hg g}^{-1}$; EnChem, Inc., Madison, WI, USA) using standard methods (USEPA, 1984; AOAC, 1990). Method blanks and standard reference material (certified dog-fish liver) were processed and analyzed concurrently with samples.

2.3. Toxicokinetic analysis

An important assumption with compartment models is that the kinetics are first-order (dose-independent; Welling, 1986). This assumption can be experimentally verified by determining the model parameters at two doses (Barron et al., 1990), and we tested this assumption in our study by administering two different dose levels in our experimental groups ($500 \mu\text{g kg}^{-1}$ oral dose and $200 \mu\text{g kg}^{-1}$ IV dose).

To determine if the elimination curves of mercury in whole blood were best described by a monoexponential (one-compartment) or a biexponential (two-compartment) elimination equation, we first described the elimination curves using the monoexponential equation:

$$C_{(t)} = Ae^{-\alpha t} \quad (1)$$

where $C_{(t)}$ is the concentration of mercury in whole blood ($\mu\text{g g}^{-1}$) at time t , and t is time elapsed since the administration of the dose (days). We then used a two-compartment model to analyze the elimination curves after dose administration for both the oral and intravenous groups. The two-compartment model is regarded as the summation of two monoexponential decay curves, one fast and one slow, and is represented by the following equation:

$$C_{(t)} = Ae^{-\alpha t} + Be^{-\beta t} \quad (2)$$

The terms A , B ($\mu\text{g g}^{-1}$) and α , β (day^{-1}) are unknown parameters to be determined by the non-linear regression model on the basis of experimental data. The variables α (one-compartment) and β (two-compartment) represent the elimination rate constant (K_{el}) of the terminal linear elimination phase of the curves. We then compared the results of our analyses to determine which model best fit the data (see statistical analyses below).

The biological half-life of mercury ($t_{1/2}$), during the elimination phase was calculated assuming

first-order elimination using the following equation:

$$t_{1/2} = \ln 2 / K_{el} \quad (3)$$

We performed non-compartmental analyses on the oral and IV blood concentration–time curves by calculating the area under the curve ($\text{AUC}_{0 \rightarrow 8}$) using the linear trapezoidal method with the area from the last sampling time to infinity calculated from the slope of the terminal portion of the concentration–time curve (Welling, 1986). An AUC was calculated for each individual within an experimental group, and the mean AUC for a group was determined as the sum of all individual AUCs divided by the number of individuals (Hothorn et al., 1994). The oral bioavailability (F) for birds dosed at age 35 days was determined using the mean AUC values for both groups as follows:

$$F = \left(\frac{\text{AUC}_{0 \rightarrow \infty} \text{ oral}}{\text{AUC}_{0 \rightarrow \infty} \text{ IV}} \right) \left(\frac{\text{dose IV}}{\text{dose oral}} \right) \quad (4)$$

We could not determine the oral bioavailability of mercury for birds dosed at day 84 using the latter approach because of the results obtained for the injected birds (see Results). We therefore estimated the percentage of the dose that was deposited in the blood volume (f), another type of bioavailability measure, as described in Kershaw et al. (1980). This measure is determined using the following equation:

$$f = \frac{(A)(V)}{D} \times 100 \quad (5)$$

where A represents the intercept of the slow component estimated from the non-linear regression models, V is the blood volume of the individual and is taken to be 7% of body mass in birds (Sturkie, 1986), and D is the dose administered orally.

2.4. Statistical analyses

We analyzed the relationship between blood mercury concentration and time using non-linear regression after having subtracted background mercury levels. Background levels for experimental birds dosed at day 35 were taken to be the levels found in same-aged control birds ($n=6$) that were consuming the same prey but received no supplemental MeHg dose. We subtracted a background level of $0.1 \mu\text{g g}^{-1}$ for all the data for birds dosed

at day 84 because this value corresponds to the steady-state level of mercury found in the blood of the six control birds (see Results). We determined the steady-state value of mercury content in whole blood of control birds sequentially sampled until day 105 by repeated-measures ANOVA.

We determined the best pharmacokinetic elimination model (monoexponential vs. biexponential) at day 35 for both intravenously and orally dosed chicks by first normalizing blood levels of each of 7 individuals to its dose, by dividing concentration of mercury in blood by dose. Second, we fit data from all seven birds to the monoexponential and biexponential elimination models and determined the residual sum of squares. Third, we used the *F*-test in Motulsky and Ransnas (1987) to determine whether the mono or biexponential model was more appropriate. After model selection, we compared the two populations (oral vs. IV doses) to see if their elimination kinetics were similar, an important assumption in pharmacokinetic modeling. To do this, we fit all individuals from each group (day 35 oral vs. day 35 IV-injected) to the same model that we previously selected. Using the residual sum of squares for the oral group alone, the injected group alone, and oral and injected groups together, we used the *F*-test in Motulsky and Ransnas (1987) to determine whether the groups were better described by a single curve or two separate curves.

We used the same approach as above to determine the best elimination model for the birds dosed at 84 days. We compared the elimination kinetics between oral groups at day 35 and day 84 to examine for age-related effects in kinetics using the same procedure that we utilized to compare the oral and injected birds at day 35.

Means are expressed \pm S.E., and we considered a *P*-value <0.05 to be statistically significant.

3. Results

3.1. Feeding, mass gain, and background mercury levels

Voluntary fish intake of loon chicks in the dosing study reached a plateau of 317 ± 23 g wet mass d^{-1} ($n=8$ loons) by 35–40 days of age, when their body mass was 2204 ± 67 g (Fig. 1, top and middle panels). The loons continued to increase in body mass beyond that age to an

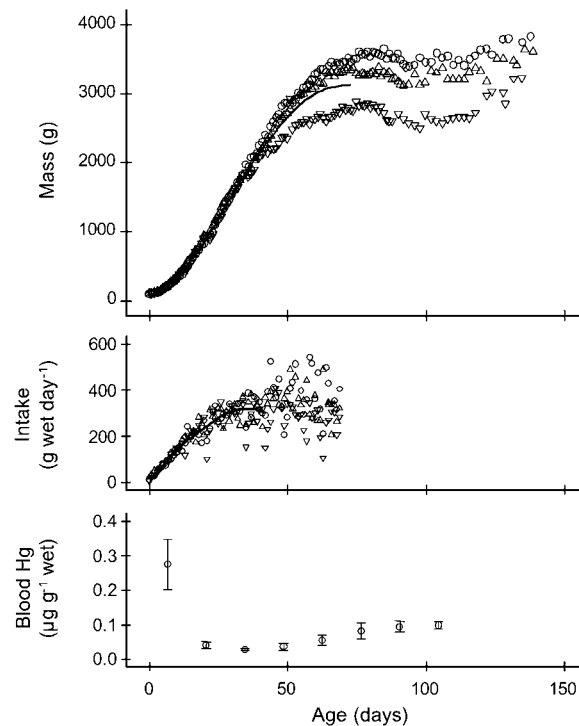


Fig. 1. Body mass (upper panel), food intake (middle panel; g wet fish d^{-1}), and background blood Hg levels (lower panel) of captive common loon chicks as a function of age. For the top and middle graphs, complete data are plotted for three individuals that were dosed with supplemental MeHg (each designated with a different symbol), whereas the lines in each graph represent the means for all 7 dosed loons. The lines are distance weighted least squares fits through the data. For the bottom graph, means \pm S.E. are shown for six control loons fed concurrently the same fish (minus dose) that the experimental loons were fed.

asymptote of 3105 ± 168 g at approximately 75 days of age.

Mercury is a naturally occurring element. All fish contain some MeHg in their tissues and thus background blood mercury levels were determined in six loon chicks fed fish but receiving no oral or IV MeHg dose. Background blood mercury level varied significantly over time (Fig. 1, bottom panel) (repeated measures ANOVA, $F_{7,35}=72$, $P<0.001$). Pairwise contrasts showed that consecutive blood Hg values differed significantly (all *P*'s <0.02) until the last comparison at days 91 vs. 105 ($F_{1,5}=0.27$, $P>0.6$).

3.2. Blood mercury levels of dosed loon chicks

Blood Hg levels of orally and IV-dosed chicks were followed for 4 and >12 weeks post admin-

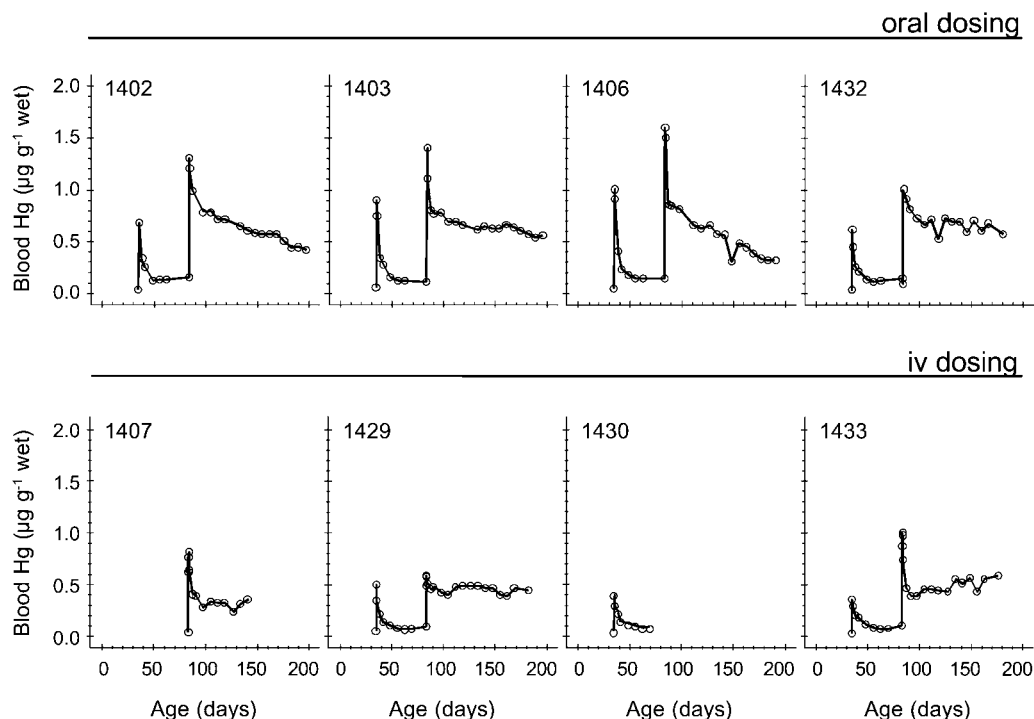


Fig. 2. Decline of blood Hg of common loon chicks following oral administration (top panel, $n=4$ birds) or IV administration (bottom panel, $n=3$ birds) of methyl mercuric chloride at ages 35 and 84 days.

istration during the first (day 35 post hatch) and second (day 84 post hatch) dosing periods, respectively (Fig. 2[WHK1]). The results for one of the birds (#1407) dosed IV at 35 days were discarded because of an analytical error during the processing of samples. Also, one of the loons in the IV-dose group (#1430) was euthanized prior to dosing at age 84 days because it developed aspergillosis.

Blood mercury rose rapidly following dosing, with peak values occurring at 2 h post IV-dosing in two birds, at 6 h post IV-dosing in a third bird, and by 8 h in 4 of 5 orally-dosed birds (no time points checked earlier for orally-dosed birds). Following the first dosing period, blood mercury levels of all dosed loon chicks declined within 4–8 weeks to those of chicks never dosed (i.e. background) (compare Fig. 1 and Fig. 2). The pattern was quite different following the second dosing period when blood Hg level declined much more slowly (Fig. 2). For this reason, the subsequent kinetic analyses were according to dosing period.

3.3. Oral bioavailability and kinetics of MeHg in 35-day-old loon chicks

Orally-dosed birds (mass 1929 ± 81 g) received 674 ± 8 μg Hg (i.e. 844 μg of methyl mercuric chloride, of which 79.9% of mass is Hg), and IV-dosed birds (1887 ± 211 g) received 272 ± 32 μg Hg. Blood mercury levels of dosed birds were normalized by subtracting appropriate age-specific background blood mercury concentrations (Fig. 1, lower panel). The relationship between post-dose blood Hg level (above background) and time post-dosing fit the monoexponential elimination model reasonably well (Fig. 3; $r^2=0.9$, $n=41$ values from the oral- and IV-dosed loons) and the fit was not significantly improved using the biexponential elimination model ($F_{2,37}=1.9$, $P>0.1$). Blood Hg values of the IV- and orally-dosed 35-day-old loons are best described by two separate monoexponential decay models ($F_{2,37}=5.42$, $P<0.05$) which differ in intercept (C_0 ; respectively, 0.39 ± 0.03 vs. 0.71 ± 0.03 μg Hg g^{-1} blood; 95% confidence intervals do not overlap) but not in elimination

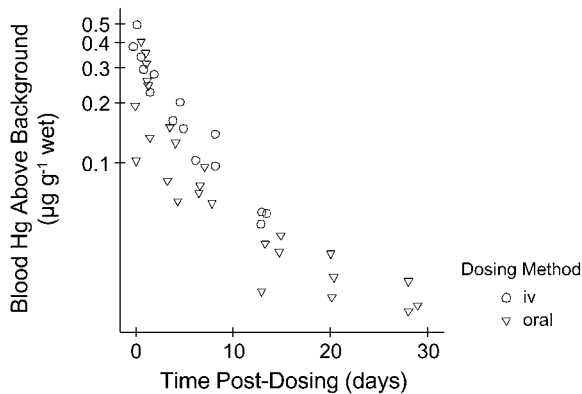


Fig. 3. Decline of blood Hg (above background) of common loon chicks following oral administration (triangles, $n=4$ birds) or IV administration (circles, $n=3$ birds) of methyl mercuric chloride at age 35 days. In order to facilitate comparison of the two groups, each value of blood Hg of the orally-dosed loons was divided by 2.48 to correct for the higher Hg dose they received compared to IV-dosed loons.

rate constant (α in Eq. (1); respectively, 0.19 ± 0.03 and $0.21 \pm 0.05 \text{ d}^{-1}$). The difference in intercept is largely due to different doses, and when normalized to dose the orally-administered loons have a 26% lower intercept, reflecting the fact that not all ingested MeHg was absorbed (see section on bioavailability, below). The similarity in slope (α) suggests that elimination was independent of mode of administration.

The mean AUC (above background) for the IV-dosed group was $2.187 \pm 0.038 \mu\text{g g}^{-1} \text{ blood}$ ($n=3$) and the mean AUC for the orally-dosed group was $4.487 \pm 0.853 \mu\text{g g}^{-1}$ ($n=4$). The latter value was higher than the former because the dose level of the orally-dosed group was 2.48 times higher than that of the IV-dosed group (above). Estimated bioavailability of orally-administered mercury was 0.83 ± 0.033 .

3.4. MeHg kinetics in 84-day-old loon chicks

At 84 days of age, birds in the orally-dosed group (mass $3293 \pm 195 \text{ g}$) received $1296 \pm 63 \mu\text{g}$ of Hg, and IV-dosed birds ($3281 \pm 324 \text{ g}$) received $530 \pm 52 \mu\text{g}$ of Hg. Just prior to this second dosing, these loon chicks had blood Hg values ($0.108 \pm 0.016 \mu\text{g g}^{-1} \text{ blood}$) very similar to background (those of loon chicks fed the same fish diet but never dosed with Hg; $0.093 \pm 0.006 \mu\text{g g}^{-1} \text{ blood}$). Because the background blood Hg concentrations were relatively constant

(0.094 ± 0.011 ; Fig. 1, bottom panel), we corrected for background following the second dosing by subtracting $0.1 \mu\text{g g}^{-1} \text{ blood}$ from every measured value. We used the same constant background Hg content beyond 105 days though actual background blood measurements were not made.

The relationship between post-dose blood Hg level and time post-dosing fit the biexponential elimination significantly better than the monoexponential elimination model ($F_{2,104}=31.9$, $P < 0.001$) for both oral and IV-dosed 84-day-old loon chicks, and elimination was much slower than when measured in 35-day-old loons. The difference is easily and dramatically visible when comparing the same four individuals dosed orally at 35 and 84 days of age (Fig. 4). The elimination half-life of the 84-day-old loons, calculated to be 116 days from the terminal slope of their elimination curves (β in Eq. (3)) = $0.0059 \pm 0.008 \text{ d}^{-1}$), is much longer than the 3-day half-life of the 35-day-old loons, calculated from the slope of their elimination curves (α in Eq. (1)) = 0.21 d^{-1} , above). Elimination of mercury was also very slow in the 84-day-old IV-dosed birds (Fig. 2), and was essentially flat in one case. Though the terminal slopes (β) for IV-dosed loons did not differ significantly from orally-dosed loons ($t_5=2.2$, $P=0.08$), they were lower, which is why we did not attempt to estimate bioavailability by comparing

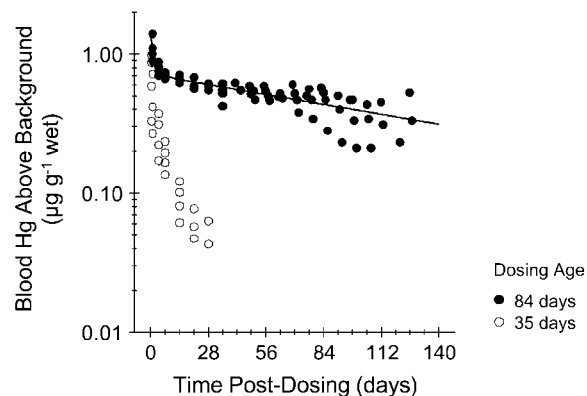


Fig. 4. Decline of blood Hg (above background) of common loon chicks following oral administration of methyl mercuric chloride at age 84 days (filled circles, $n=4$ birds). The solid line is the best fit to the biexponential decay model (see Results): $\text{blood Hg} = 0.64 (\pm 0.13) \exp^{-0.47 (\pm 0.18) \cdot \text{time}} + 0.71 (\pm 0.03) \exp^{-0.0059 (\pm 0.008) \cdot \text{time}}$ ($r^2=0.98$). Shown also, for comparison, are blood Hg values of the same four loons when they were dosed at age 35 days (open circles, from Fig. 3).

the orally-dosed and IV-dosed loons during the second dosing period.

4. Discussion

The absorption of ingested MeHg in loon chicks, expressed as a sharp rise in blood Hg content, was rapid at both dosing periods (<8 h) and is comparable to results found in humans (<10 h; Kershaw et al., 1980).

The oral bioavailability of ingested MeHg measured in chicks dosed at day 35 was 83% and is similar to values found for other endothermic vertebrates. For example, the oral bioavailability of MeHg is generally high in laboratory mammals such as rats (>90%, Farris et al., 1993; Carrier et al., 2001). In fish, bioavailability seems species-specific and varies more widely, between 33 and 80% or greater (Weiner and Spry, 1996; McCloskey et al., 1998). [ecv3] We expect that our value of 83% probably reflects the oral bioavailability of MeHg for wild loons because the latter consume similar types of food as we used in our dosing trials. Because we could not directly measure oral bioavailability in older chicks due to the difficulty in using results of the IV-dosed chicks (see below), we compared oral bioavailability at days 35 and 84 by using another estimate of bioavailability which is defined as the percentage of the ingested dose deposited in the blood volume (f , Kershaw et al., 1980). We calculated this value at both ages and found that the results were very similar between 35-day-old and 84-day-old loon chicks (5.8 vs. 5.2%, respectively [ecv4]), therefore suggesting similar oral bioavailabilities. The similarity in these calculations taken together with the fact that the rapid rise in blood Hg content was similar between oral dosing periods (see above) strongly suggests that oral bioavailability did not differ between dosing periods. Our estimates of the percentage of ingested Hg deposited in the blood are comparable to values found for humans (5.9 and 5.6%; Kershaw et al., 1980; Sherlock et al., 1984, respectively [ecv5]), but somewhat different than that for adult Cory's shearwater (*Calonectris diomedea*) (10.4%; Monteiro and Furness, 2001a). Monteiro and Furness suggest that the higher deposition of Hg in the blood of shearwaters may be the result of several factors such as higher rates of intake in shearwaters, species-specific differences in the ratio between MeHg in red blood cells and plasma, and species-specific differences

in the behavior of MeHg in erythrocytes (Monteiro and Furness, 2001a).

Therefore, a very high proportion (83%) of the ingested MeHg is absorbed into the blood and is distributed to other tissues in the body. The other major mechanism that will affect an individual's burden and possibly the toxicity of the contaminant will be the rate at which the latter is eliminated from the body. This is an important point for growing organisms because they may be more susceptible to the deleterious effects of contaminants [ecv6].

The elimination of Hg was much slower when chicks were older than when they were dosed at a younger age. This conclusion is apparent to the eye in the figures and very robust statistically. As previously mentioned, the absorption of orally administered MeHg was rapid (<8 h) followed by a sharp decline in blood Hg content. The elimination half-life for this dosing period was 3 days, and the Hg content in blood fell close to background levels within 4 weeks post-administration. This would suggest that any MeHg absorbed by an individual at this age would be distributed and eliminated quite rapidly. Loon chicks start replacing their first down feathers with secondary down feathers immediately upon hatch, and they can also eliminate Hg through down feathers before hatching. The elimination of Hg through feathers continues until the completion of flight feathers at approximately 11 weeks (McIntyre and Barr, 1997). This period of intense feather growth corresponds to the same time period when the birds received their first dose. In comparison, the second dose was administered after this period of intense feather growth and it was eliminated much more slowly, with a half-life at least 25 times longer. Therefore, the comparison of elimination of the two doses highlights the importance of feathers as a major elimination pathway in growing birds.

Similar results as those reported in the present study were found in a study of the toxicokinetics of methylmercury in Cory's shearwater chicks (Monteiro and Furness, 2001b). Shearwater chicks dosed at two intervals during feather growth had a similar pattern of mercury uptake and elimination, characterized by a short half-life of mercury (range 5.5–6.3 days; Monteiro and Furness, 2001b). The latter results are very different from half-life estimates in adults (40–60 days; Monteiro and Furness, 2001a), and for older loon chicks

dosed after the completion of feather development (116 days). Both studies highlight the importance of sequestering mercury into growing feathers as an effective excretion mechanism.

Along with feathers, the major routes for elimination of MeHg in birds are the feces and urine, and eggs in females (Braune and Gaskin, 1987a; Lewis and Furness, 1991; Lewis et al., 1993). Sequestration of MeHg into growing feathers has been shown to be an important excretory pathway in birds. Between 33% to 93% of the total body burden of Hg has been found in feathers (Braune and Gaskin, 1987a,b; Braune, 1987; Lewis et al., 1993; Monteiro and Furness, 2001a,b). Our results strongly suggest that ingested MeHg is rapidly excreted to growing feathers (day 35), and that once feather development is complete (day 84) its elimination is much slower through another excretory pathway (probably through urine and or feces). The relatively rapid elimination of a major proportion of the ingested MeHg by younger chicks growing feathers may act to buffer young birds from the deleterious effects of MeHg at an age when they are not physiologically or immunologically mature (Apanius, 1998).

The kinetics of MeHg for birds dosed at 35 days of age were best fitted by a one-compartment monoexponential decay model, whereas the kinetics of MeHg for birds dosed at age 84 days were best described by a two-compartment elimination model. Both models have been described in past studies. The one-compartment model of elimination that we found for loon chicks dosed at 35 days is similar to the kinetics of MeHg for orally-dosed human subjects (Smith et al., 1994; Smith and Farris, 1996; Carrier et al., 2001). Two-compartment models have been applied to rats (Farris et al., 1993) and adult Cory's shearwaters (Bearhop et al., 2000; Monteiro and Furness, 2001a). It has been suggested that a possible explanation for the two-compartment model for rats is the result of a feedback loop following the ingestion of hair during grooming (Farris et al., 1993; Carrier et al., 2001), because Hg that was excreted to hair during growth re-entered the body pool (Farris et al., 1993). We doubt that this occurs in birds, which do not consume feathers during grooming. In the older loons and adult Cory's shearwaters, the rapid decline in blood Hg post-dosing may reflect distribution to other peripheral compartment(s), followed by slower elimination from the central compartment via lim-

ited deposition into feathers and excretion in feces and urine. Alternatively, Bearhop et al. (2000) state that Hg profiles in blood and feathers were strongly correlated throughout their study, suggesting a rapid transfer of Hg from blood to feathers, and that the initial decay in the model may reflect this process. Arguably, more sampling points in the younger loons dosed at 35 days may have provided resolution necessary to fit a dual exponential decay model. We do not think that uncertainties about the exact identification of putative compartments in younger vs. older loons confounds our overall conclusion that feather growth in the younger loons greatly increased the rate of elimination of Hg from their blood in comparison with older loons not growing feathers.

This age-related shift in rate of elimination may have important implications for equilibration times with environmental MeHg level, and may also impact on loon blood Hg levels at steady state. Our results suggest that blood Hg level would be relatively low during feather development, but that it would rise after the completion of feather growth. Blood levels could therefore reach a steady state which would indicate an equilibrium between intake and elimination. For example, the concentration of Hg in the blood of captive shearwaters outside the molt increased steadily and seemed to reach a plateau even though their food contained very low amounts of Hg (Bearhop et al., 2000). We also found the same pattern of rising blood levels of Hg in six control common loon chicks fed the same food resources as the experimental birds but never receiving a supplemental dose of MeHg (Fig. 1, lower panel). Notably, this occurred after feather growth ceased and the rate of Hg elimination greatly declined.

The initial blood level in the control chicks was high and was probably a residual of *in ovo* transfer of Hg from the female to the chick during egg formation (Lewis and Furness, 1993; Lewis et al., 1993; Monteiro and Furness, 2001a). This level rapidly declined due to elimination and perhaps growth dilution, and remained low until approximately week seven at which time it started to rise and it reached an asymptote at approximately week 13-post hatch. We do not think that the Hg level in the blood of the control chicks rose after week seven due to greater ingestion of Hg because food intake and fish Hg content both remained constant, but because the elimination rate declined following the termination of feather development (refer to

discussion above), and ultimately resulted in raising the steady state level. Our results lend empirical support to the suggestion that mercury accumulates in the body tissues during the intermolt period as a result of the primary excretory pathway (feathers) being no longer available to the individual (Furness et al., 1986; Bearhop et al., 2000).

We had mixed results following the intravenous dosing of mercury. The post-dosing elimination results for the IV-dosed 35-day-old loons corresponded quite well to the orally-dosed birds, and the rate constants that we obtained for both data sets did not differ, an important assumption in toxicokinetic modeling. However, the results we obtained following the IV dosing of mercury in the 84-day-old chicks were unexpected. It is unclear why blood concentrations ceased to decline in some individuals, while blood levels in orally-dosed chicks showed a typical elimination pattern. This result invites speculation about complicated kinetics, for example involving the effect of first-pass liver processing (affects oral-dosed birds but not IV-dosed birds, Gibaldi and Perrier, 1974), but this is beyond the scope of this paper. For ecological purposes the intravenous results are an artifact because wild birds don't get dosed IV, and it was shown that bioavailability was similar at both periods.

What do all of these results mean for wild loon chicks? In nature loon chicks receive their first dose of MeHg as developing embryos because it is transferred from the female during egg formation. This may be the most critical stage for toxicity not only for loons but also for most species because of the neurological effects of mercury, but so far no dose–response study has been conducted on eggs. Reproductive failure in loons has been correlated with water chemistry, and hence prey mercury content (Meyer et al., 1998), but it is unclear if this is due to in ovo exposure. For those chicks that do hatch, they almost immediately begin to consume prey items that contain MeHg, and they can eliminate the latter through different pathways (feces, feather growth, etc.). During feather growth this excretory route becomes the primary way individuals rid themselves of ingested MeHg, and we have shown that they eliminate Hg quite rapidly during this period. Blood Hg levels during this rapid elimination phase should remain low and will depend on MeHg content in the food items that they consume. At the time that feathers

stop growing the Hg content in blood should start to rise until it reaches a steady state between intake and elimination, and this plateau will also depend on MeHg content in food and it will probably be much higher in these older loons because of their much slower rate of elimination. Although molting offers adult birds an opportunity to reduce their body burden in contaminants, juvenile loons, unlike adults, do not molt during their first autumn but begin molting on the wintering grounds following the fall migration [ecv8](McIntyre and Barr, 1997). This suggests that the body burden of juveniles rises on their natal lakes after feather growth and possibly reaches an equilibrium, but that it may also continue to rise after migration depending on the location where they overwinter and the concentration of MeHg of prey on these wintering areas. Adults molt following the breeding season, therefore prior to the migration, and can thus reduce their body burden before their arrival on the wintering areas (McIntyre and Barr, 1997). We could therefore expect that juveniles will have the highest body burden on a per gram basis. This would lead us to speculate that the most critical periods for loons are probably first during exposure in ovo, and second during the overwintering period of their first year.

Our results on the toxicokinetics of mercury in loons help us to understand better the effects that Hg may have on wild loons. Those effects are probably subtle and involve low level toxicity associated with behavioral modifications in chicks (Nocera and Taylor, 1998), and reproductive failure in adults nesting on lakes containing prey with high MeHg content (Meyer et al., 1998).

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