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METHYL MERCURY TOXICOKINETICS IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) AND LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*) AFTER INTRAVASCULAR ADMINISTRATION

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Abstract—We compared the differences in the distribution and elimination of CH_3Hg after intraarterial injection and serial blood removal in catfish and bass of similar body size under consistent water quality conditions. The blood and plasma concentration–time profiles of individual fish were analyzed using a three-compartment, clearance–volume model. The plasma protein binding of CH_3Hg was determined by ultrafiltration (30,000 mol. wt. cutoff) and the binding affinity (ρ) of CH_3Hg for red blood cells (RBCs) was also calculated. Toxicokinetic analysis of the plasma concentration–time profiles provided the following values: apparent volume of distribution at steady state (V_{ss}) = 30 ± 14 ml/g (catfish), 6.2 ± 2 ml/g (bass); total body clearance (Cl_b) = 0.026 ± 0.011 ml/h/g (catfish), 0.0057 ± 0.001 ml/h/g (bass). The values of V_{ss} and Cl_b estimated from the blood concentration–time profiles in catfish and bass were fivefold lower. The elimination half-life from blood and plasma was between 814 and 1670 h and was not statistically different between species or reference fluid. The $AUC_{0\rightarrow\infty}$ for blood was over three times higher than plasma, due to the binding of CH_3Hg to RBCs. The unbound fraction of CH_3Hg in bass plasma was 14-fold lower (0.25 vs. 3.64%) and the ρ for RBCs was 20 times greater than catfish (5,974 vs. 289). The decreased binding to plasma and RBCs in catfish is consistent with the increased extravascular distribution and clearance capacity of CH_3Hg in catfish because a larger fraction of the CH_3Hg in blood is available to distribute outside the vascular system.

Keywords—Methyl mercury Toxicokinetics Catfish Bass Plasma binding

INTRODUCTION

Mercury input to aquatic environments from industrial sources will increase into the next century [1,2]. As a result, the concentration of Hg in many aquatic organisms will probably continue to increase. The primary chemical form of Hg found in aquatic organisms is the highly toxic methyl mercury (CH_3Hg) [3]. This represents both an ecological and human health concern because consumption of fin and shellfish is the primary source of Hg to humans and many piscivorous wildlife [4–8]. A recent survey of state fish consumption advisories found that Hg was the most commonly cited pollutant, accounting for 60% of all advisories [9].

The accumulation of CH_3Hg in fish has been studied after water and oral exposures [10–12]. Methyl mercury is rapidly absorbed from water by rainbow trout (*Oncorhynchus mykiss*) with an uptake efficiency of 8% [13]. The assimilation of CH_3Hg from food also proceeds with high efficiency and has been reported to be 70 to 90% in goldfish (*Carassius auratus*) [14] and greater than 73% in rainbow trout [15]. The elimination of CH_3Hg from fish is slow with biological half-lives ranging from 16 to 1,000 days [12]. The long elimination half-life for CH_3Hg has been attributed primarily to its accumulation and persistence in skeletal muscle [15]. A characteristic of CH_3Hg distribution that can influence its elimination is its binding to red blood cells (RBCs). The RBC-to-plasma ratio of CH_3Hg measured after in vitro incubation with rainbow trout blood is 9 [16] and is similar to values reported for humans [17], although substantial interspecies differences exist among other mammalian species [18].

The influence of environmental and biological variables (e.g., body size, age, acclimation temperature, water quality, and pH) on the accumulation of Hg in fish has been reported in the literature. In general, larger, older fish in low alkaline, low pH lakes tend to accumulate the highest concentrations of Hg [4,14,19,20]. Mercury body burden can vary widely among fish species within a lake [21], and this difference is often attributed to the trophic level of the species, with top predators such as largemouth bass (*Micropterus salmoides*) accumulating more Hg than forage species [21,22].

A less-studied aspect of mercury accumulation in fish is the importance of intrinsic differences among fish species, unrelated to trophic level. These differences may also influence the accumulation or clearance of Hg. The present study compares the differences in the distribution and elimination of CH_3Hg after intravascular administration to channel catfish (*Ictalurus punctatus*) and largemouth bass of similar body size under consistent water quality conditions. The plasma protein and red blood cell binding and the total lipid content of the catfish and bass are also reported to ascertain if they can be used to explain differences in the toxicokinetics between catfish and bass.

MATERIALS AND METHODS

Fish and water quality

Channel catfish (body weight ± SD: 755 ± 80 g) of mixed sex were obtained from Orangeburg Aquaculture (Cordova, SC, USA). Largemouth bass (696 ± 130 g) were collected by hook and line from Par Pond, a reservoir located on the U.S.

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Department of Energy's Savannah River Site, South Carolina, USA. Immediately upon arrival at the laboratory, all fish received a 2-h treatment in a 0.25-mg/L solution of malachite green (Sigma Chemical, St. Louis, MO, USA). Catfish were held indoors in 400- and 600-L, recirculating water, fiberglass aquaria (LS 700, LS 900, Frigid Units, Toledo, OH, USA) containing reconstituted hard water [23] and 1‰ (w/v) NaCl. Bass were initially held outdoors in 2,800-L, polyethylene-lined, circular tanks for a minimum of 2 weeks before transfer to the indoor aquaria. After transfer indoors, all fish were held at 21°C for a minimum of 2 weeks prior to use in experiments. The loading density of fish in all aquaria was maintained below 5 g/L. Half of the aquarium water was replaced biweekly. Chemical characteristics of the freshly prepared water were: total alkalinity 110 to 120 mg/L (as $CaCO_3$), hardness 160 to 180 mg/L (as $CaCO_3$), and pH 7.9. Temperature and pH were monitored daily and ranged from 20 to 22°C and 7.7 to 7.9, respectively. Ammonia levels were regularly monitored to ensure that the concentration remained below 0.5 mg/L. Catfish were fed a maintenance ration of approximately 2% of their body mass three times per week with soft moist pelleted feed (Rangen Inc., Buhl, ID, USA). Bass were fed live minnows two to three times per week.

Surgical procedures and blood removal

Each fish was fitted with a dorsal aortic cannula using methods described previously [24,25]. Catfish were anesthetized with 150 ml/L MS-222, largemouth bass with 100 mg/L MS-222. The cannula material was 28-G Teflon tubing (Zeus Inc., Raritan, NJ, USA). An 18-G intravenous catheter (Angiocath®, Becton Dickinson, Sandy, UT, USA) was used to guide the cannula into the dorsal aorta. The cannulated fish were held in 100-L polyethylene cages that were perforated to allow water exchange. The cages containing cannulated fish were placed in round, recirculating water, 1,000-L plastic aquaria filled with reconstituted hard water and 1‰ (w/v) NaCl. The end of the cannula was attached to a 1-ml syringe that floated above the fish inside the cage. All fish were allowed to recover from surgery for a minimum of 24 h before dosing.

Immediately prior to injection with CH_3Hg, a blood sample was removed for determination of hematocrit (hct) and background CH_3Hg concentrations in blood and plasma. Hematocrit was determined using heparinized microhematocrit tubes. Hematocrit was determined for all blood samples removed after CH_3Hg injection, beginning with the 4-h sample.

Catfish and bass received a 0.47-mg/kg intraarterial (IA) bolus injection of CH_3HgCl_2 (purity >99%, ICN Biomedicals, Inc., Costa Mesa, CA, USA) dissolved in 0.9% NaCl and 5 mM sodium carbonate. This dose was chosen after preliminary studies revealed that a higher dose of 1 mg/kg produced necrotic lesions around the trunk kidney and posterior skeletal muscle regions of the fish. These lesions typically appeared between 100 and 400 h after IA injection. The lower dose of 0.47 mg/kg produced no visible signs of toxicity in catfish and bass. After CH_3Hg injection, serial blood samples were removed via the cannula at 0.167, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 6, 9, 12, 24, 36 (catfish only), 50, 75, 100, 125, 200, and 300 h, and replaced with an equal volume of a modified teleost saline [26]. The volume of blood removed for each sample varied between 0.1 and 0.25 ml. The cannula was removed while the fish was under anesthesia after the 300-h sample collection. Later blood samples were obtained from the dorsal aorta of anesthetized catfish and bass using a 25-G needle attached to a 1-ml syringe. The body weight of the fish was also recorded at this time. After 300 h, the sampling interval for blood removal was increased to approximately 250 h because CH_3Hg concentrations in blood and plasma were declining slowly. Sampling continued for 3,000 h (125 d) for catfish and 1,500 h (62 d) for bass. The catfish and bass were not fed during the initial 300-h sampling period. Thereafter, catfish were fed approximately 2% of their body mass 3 d per week with the pelleted feed. This ration level was sufficient to maintain the catfish within 90% of their initial body weight. We attempted to feed the bass three times per week with live minnows. However, some individuals would not consistently feed and the final body weight of the nine bass used in this study was 82 ± 8% (mean ± SD) of the initial body weight. Blood samples were not removed on days that fish were fed. The cumulative volume of blood removed via the cannula was less than 10% of the estimated blood volume of the fish (assumed to be 4% of body weight). After resumption of feeding, the removal of blood was limited to 0.40 to 0.75 ml per 250-h sample interval, which was less than 2% of the estimated blood volume.

Analytical procedures

Both whole blood and plasma (obtained by centrifuging blood at 2,000 *g* for 5 min) were assayed for CH_3Hg using a Perkin-Elmer (model 5100ZL) graphite furnace atomic absorption spectrometry (GFAAS) and a modification of methods described by Filippelli [27]. Briefly, aliquots of fresh blood or plasma (maximum sample volumes of 0.75 ml) were mixed with an equal volume of a 1% NaCl (w/v) and 2 N HCl solution, and immediately frozen. Later, the samples were thawed, and the CH_3Hg was removed by quadruplicate extractions with 300 μl of benzene. The benzene extracts were pooled and then extracted with 500 μl of 2.5 mM $Na_2S_2O_4$ (>99.999% pure, Aldrich Chemical, Milwaukee, WI, USA). The $Na_2S_2O_4$ layer was removed and the CH_3Hg concentration determined by GFAAS. A standard curve of CH_3Hg in 2.5 mM $Na_2S_2O_4$ was prepared each day of analysis. A set of three blood or plasma standards, spiked with a known amount of CH_3Hg to encompass the expected concentration range of unknown samples, was prepared and frozen as described previously. These standards were thawed and assayed simultaneously with experimental samples. The recovery of CH_3Hg from spiked samples was (mean ± SD) blood, 93 ± 7%, n = 15; plasma; 95 ± 13%, n = 62. The calculated CH_3Hg concentrations were not corrected for recovery because of the consistently high recovery of CH_3Hg from spiked standards.

The blood sampling protocol was chosen due to limitations on the volume of blood that can be removed from the fish and as a compromise between the needs for frequent sampling to fully characterize the blood/plasma concentration–time profiles and high precision in the estimation of blood and plasma CH_3Hg concentrations. The final protocol was based, in part, on results of an experiment performed to determine if any advantage in analytical precision could be obtained by removing replicate blood samples at fewer sample times. A single catfish (body wt. = 950 g) was administered a 0.47-mg/kg IA dose of CH_3Hg as described previously. At 100 h postinjection, five sequential 1-ml blood samples were rapidly removed using separate, 1-ml syringes. After removal of each 1-ml sample, the cannula was rinsed with heparinized saline, then blood was redrawn into the cannula and the first 50 μl discarded before removal of the next sample. Next, plasma

was obtained from each blood sample and three equal volume aliquots were separated and assayed for CH_3Hg as described previously. A nested ANOVA was performed to determine the variability associated with sample removal, extraction of CH_3Hg from plasma, and GFAAS analytical steps. Minimal (<10%) variation among plasma samples indicated there was no advantage to taking more than one sample at each sampling time.

The hct of blood samples removed via the cannula during the initial 300-h sampling period were typically less than the hct of later blood samples removed after anesthetizing the fish. This observation is consistent with previous reports indicating that the blood sampling protocol influences the hct [28]. Because the majority of CH_3Hg in blood was associated with the RBC fraction, we adjusted the blood concentrations of CH_3Hg of individual fish to the hct value determined prior to injection by the following equation:

$$C_{adj} = (C_{RBC}/\text{hct})\cdot\text{hct}_{init} + [C_p\cdot(1 - \text{hct}_{init})]$$

where C_{adj} = adjusted blood concentration, C_{RBC} = the difference between the observed CH_3Hg concentrations in blood and plasma, hct = the observed hematocrit of the blood sample, hct_{init} = the hct of blood prior to injection, and C_p = the observed plasma concentration. This adjustment assumes that actual changes in hct due to blood removal during the experiment are similar between the two species.

Plasma protein and blood binding

Plasma protein binding was determined using the Centrifree micropartition system (mol. wt. cutoff 30,000; Amicon Inc., Beverly, MA, USA). Plasma samples (0.55 ml) from each species were combined with 1 μg CH_3Hg (dissolved in 10 μl 0.9% w/v, NaCl) and incubated at 21°C for 20 min. After incubation, a 50-μl aliquot was removed to determine plasma CH_3Hg concentrations. The remaining plasma was pipetted into Centrifree units and centrifuged at 1,500 *g* for 15 min. The ultrafiltrate (100–150 μl) and the plasma aliquot were analyzed for CH_3Hg as described previously. Calculation of the unbound fraction was made using the following equation:

$$\%\ \text{unbound} = ([CH_3Hg]\text{ultrafiltrate}/[CH_3Hg]\text{plasma}) \times 100$$

The binding of CH_3Hg to the ultrafiltration membrane was determined to be less than 10%.

Calculation of the affinity constant of CH_3Hg for RBCs (ρ) was made using the following equation [29]:

$$\rho = [(\text{hct} - 1) + (C_b/C_p)]/(f_u\cdot\text{hct}) \quad (1)$$

where f_u = the unbound plasma fraction of CH_3Hg, and C_b and C_p are the observed blood and plasma concentrations of CH_3Hg, respectively. The ratios of the $AUC_{0\to\infty}$blood/$AUC_{0\to\infty}$plasma were used to estimate C_b/C_p.

Lipid content of catfish and bass

The nonpolar lipid content of catfish and bass used in this study was determined by Soxhlet extraction of dried fish sample [30]. The fish carcass was homogenized with an equal volume of water using a Waring blender, and a 50-g aliquot was freeze dried. Three grams (dry weight) of the freeze-dried samples was used for nonpolar lipid extraction. Nonpolar lipids were extracted for a minimum of 5 h using petroleum ether. The mass of nonpolar lipids in each sample was calculated as the difference in sample dry mass before and after extraction.

Toxicokinetic analysis

An iterative, nonlinear least-squares computer program, PCNONLIN (Statistical Consultants, Inc., Lexington, KY, USA) was used to fit the blood and plasma concentration time (C_bt, C_pt) profiles of CH_3Hg after IA injection. Preliminary analysis of the C_bt, C_pt profiles using a two- or three-compartment pharmacokinetic model indicated the three-compartment model provided a better fit to the observed data based on several criteria: Akaike information criterion, coefficients of variation, and visual inspection of the fits [31,32]. The following triexponential equation was used to fit the observed blood and plasma concentration–time profiles:

$$C_bt,\ C_pt = Ae^{-\pi t} + Be^{-\alpha t} + Ce^{-\beta t} \quad (2)$$

The area under the curve ($AUC_{0\to\infty}$), was estimated using the following equation: $AUC_{0\to\infty} = A/\pi + B/\alpha + C/\beta$. The biological half-life ($t_{½,\beta}$), mean residence time (MRT), area under the moment curve (AUMC), total body clearance (Cl_b), and apparent volume of distribution at steady state (V_{ss}) were estimated by the following equations: $t_{½,\beta} = 0.693/\beta$, MRT = $AUMC/AUC_{0\to\infty}$, where $AUMC = A/\pi^2 + B/\alpha^2 + C/\beta^2$, Cl_b = dose/$AUC_{0\to\infty}$, $V_{ss} = Cl_b\cdot MRT$.

The fraction of the injected dose remaining in the fish (X_f) was estimated by the following equation [33]:

$$X_f = \frac{(A/\pi)e^{-\pi t} + (B/\alpha)e^{-\alpha t} + (C/\beta)e^{-\beta t}}{A/\pi + B/\alpha + C/\beta} \quad (3)$$

A simulation comparing the fraction of the injected dose of CH_3Hg remaining in catfish and bass was performed using Equation 3 and the values of *A*, *B*, *C*, π, α, and β determined from a fit of the averaged blood concentration–time profiles for catfish and bass.

Student's *t* test was used to test for significant differences between parameter values after using Cochran's test to determine if the variances among classes were unequal.

RESULTS

Toxicokinetic analysis of CH_3Hg in channel catfish and largemouth bass

The elimination of CH_3Hg from blood and plasma was qualitatively similar for catfish and bass. After injection, the concentrations of CH_3Hg in blood and plasma declined rapidly during the first 12 h and then decreased more slowly (Figs. 1 and 2). After 125 h, the CH_3Hg concentrations began to decline in an apparent, log-linear fashion. Immediately after injection (0.167 h), the concentration of CH_3Hg in whole blood was three- to sevenfold higher than in plasma, indicating greater accumulation of CH_3Hg by blood cells (Figs. 1 and 2). The CH_3Hg concentration ratio for blood and plasma remained relatively constant throughout the experiment and indicated that equilibrium of CH_3Hg was rapidly achieved between blood cells and plasma. No detectable CH_3Hg was observed in catfish blood or plasma prior to injection. In bass, CH_3Hg could not be detected in plasma; however, some fish had preinjection blood concentrations of CH_3Hg between 1 and 2 ng/ml.

The parameter estimates from the nonlinear, least-squares fitting of the data are shown in Table 1. The two pharmacokinetic parameters that are particularly useful in understanding the distribution and elimination of CH_3Hg are the apparent volume of distribution at steady-state (V_{ss}) and total body clearance (Cl_b). The V_{ss} relates the amount of CH_3Hg in the fish to the concentration in the reference fluid (blood or plasma) when

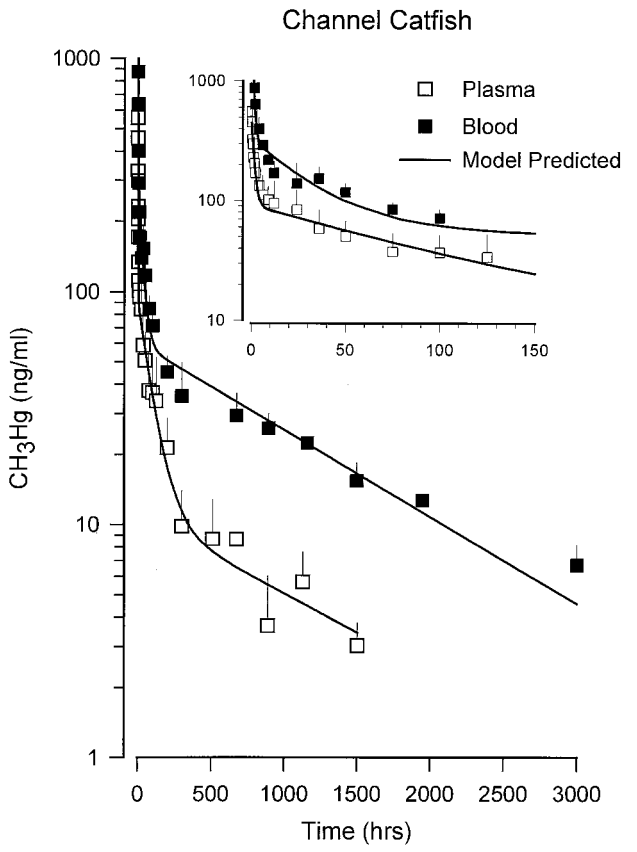

Fig. 1. The CH_3Hg plasma (□) and blood (■) concentration–time profiles after intraarterial injection of 0.47 mg/kg CH_3Hg in channel catfish. Symbols represent experimentally determined values (mean ± SD, n = 4–6) and the line is the least-squares fit to Equation 2. Error bars not shown fit within the data point.

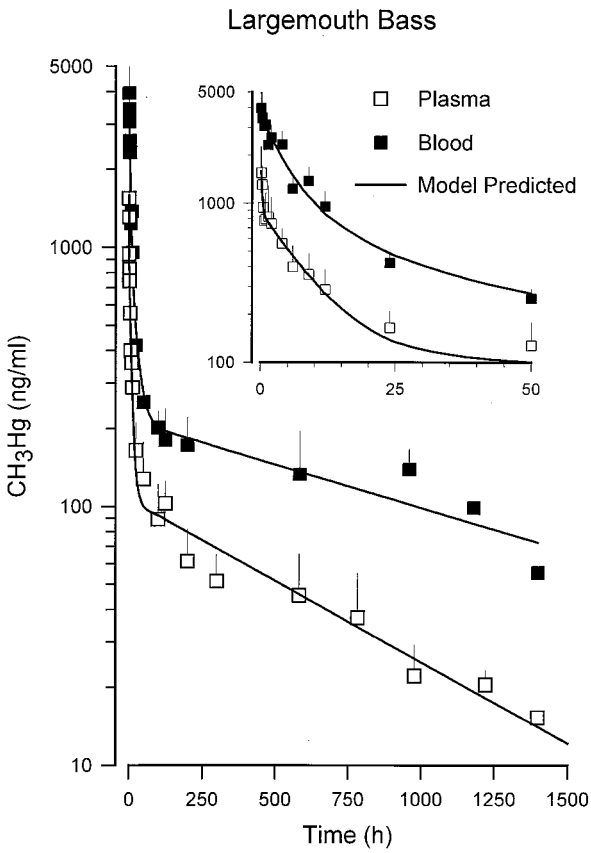

Fig. 2. The CH_3Hg plasma (□) and blood (■) concentration–time profiles after intraarterial injection of 0.47 mg/kg CH_3Hg in largemouth bass. Symbols represent experimentally determined values (mean ± SD, n = 5–8) and the line is the least-squares fit to Equation 2. Error bars not shown fit within the data point.

an equilibrium exists between peripheral tissues and the reference fluid (i.e., steady-state conditions). The very large V_{ss} in catfish and bass relative to blood volume (ca. 4% body weight) [34] indicated that CH_3Hg was extensively distributing outside the vascular system (Table 1). The Cl_b represents the sum of all elimination pathways (i.e., urinary, fecal, branchial, and metabolic) of CH_3Hg from catfish and bass and can be interpreted as the volume (ml) of blood or plasma that is completely cleared of CH_3Hg per hour. Thus, the small Cl_b (Table 1) relative to cardiac output (0.06–0.7%) in catfish [28] and other teleosts [35,36] indicated only a negligible quantity of CH_3Hg is excreted relative to the flow rate of blood through

Table 1. Toxicokinetic parameters determined from the plasma and blood concentration–time profiles of channel catfish and largemouth bass

	Channel catfish[a]		Largemouth bass[b]	
Parameter	Plasma	Blood	Plasma	Blood
$AUC_{0\to\infty}$ (ng/ml h)	23,300 ± 7,200**	77,500 ± 9,100**	97,600 ± 34,200	348,600 ± 84,000
C_{max} (ng/ml)	624 ± 47	6,050 ± 1,950	2,361 ± 382	4,400 ± 450
V_{ss} (ml/g)	30 ± 14**	6.2 ± 1*	6.2 ± 2	1.6 ± 0.1
Cl_b (ml/h/g)	0.0260 ± 0.0110*	0.0059 ± 0.0001**	0.0057 ± 0.001	0.0014 ± 0.0004
$t_{1/2}$ (h)	1,670 ± 683	814 ± 58	905 ± 189	1,008 ± 275
MRT (h)	1,829 ± 953	1,046 ± 77	1,054 ± 324	1,261 ± 349
f_u (%)[c]	3.64 ± 0.35		0.25 ± 0.02	
ρ[d]		289		5,974

Catfish and bass were administered a 0.47-mg/kg intraarterial dose of CH_3Hg. Parameter estimates were calculated by the PCNONLIN program after fitting the individual blood and plasma concentration–time profiles to Equation 2 to obtain estimates of A, B, C, π, α, and β. The mean ± SD is presented for the following samples sizes: catfish, n = 6 plasma, n = 4 blood; bass, n = 8 plasma, n = 5 blood. Asterisks indicate values significantly different from bass: * $p < 0.05$; ** $p < 0.01$.

[a] Mean body weight (±SD) = 755 ± 80 g.

[b] Mean body weight (±SD) = 696 ± 130 g.

[c] f_u is the percentage of CH_3Hg that is unbound to plasma proteins and was determined by ultrafiltration of plasma through a 30,000-mol. wt. cutoff membrane.

[d] ρ is the affinity constant for CH_3Hg to RBCs as calculated with Equation 1.

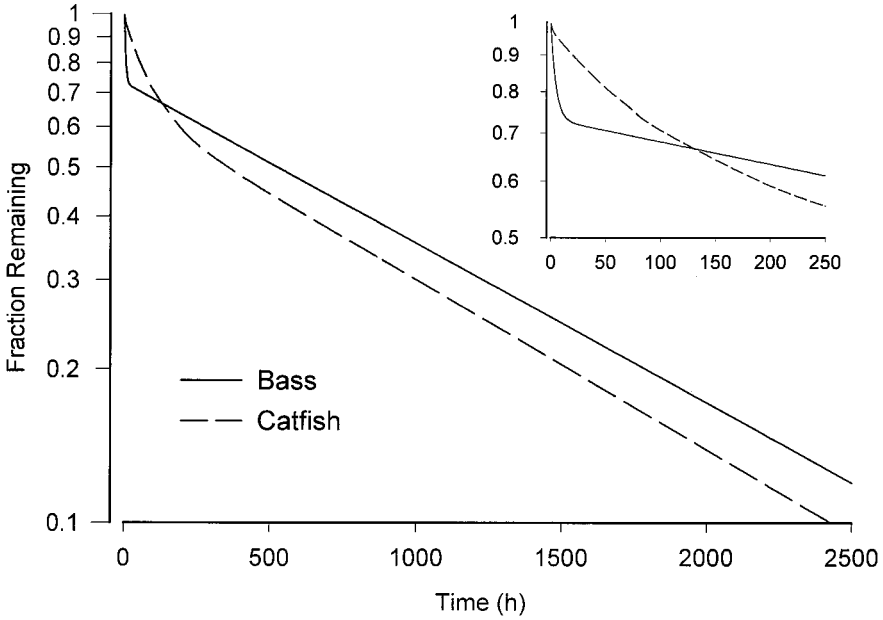

Fig. 3. Simulation depicting the fraction of an intraarterial injected dose of CH_3Hg remaining in the fish. The fraction remaining was calculated using Equation 3 and the values of *A*, *B*, *C*, π, α, and β determined from the fit of the averaged blood concentration–time profiles shown in Figures 1 and 2.

eliminating organs. It is the combination of a large V_{ss} and small Cl_b that causes the long biological half-life of CH_3Hg (>34 d; Table 1), whether estimated from the blood or plasma concentration–time profiles.

The effects of binding to blood cells by CH_3Hg was evident in several parameter estimates. The AUC for blood was three to four times higher than plasma in both catfish and bass, and the estimates of the V_{ss} and Cl_b were approximately fourfold lower from blood (Table 1). The $t_{½,\beta}$ and MRT were similar in catfish and bass although the plasma $t_{½,\beta}$ estimated for catfish was higher, but not significantly so (Table 1).

The most statistically significant differences in parameter estimates between catfish and bass were the larger V_{ss} and higher Cl_b observed in catfish (Table 1).The plasma referenced estimates of the V_{ss} and Cl_b were 4.8 and 4.5 times larger in catfish compared to bass, respectively (Table 1). These differences indicated that in catfish, CH_3Hg was concentrated to a greater extent in peripheral tissues, and that catfish have a greater capacity for elimination of CH_3Hg compared to bass. A simulation of the fraction of an IA dose of CH_3Hg remaining in the fish is shown in Figure 3. The simulation predicts that initially a greater fraction of the injected dose is retained by catfish, but later (>250 h), a larger fraction is retained by bass.

The mean lipid contents of catfish and bass used in this study were 3.6 ± 0.8 and 1.4 ± 0.7%, respectively. Cursory examination of these data indicated no obvious correlation between lipid content and differences in the toxicokinetic parameters.

Plasma and blood binding

There were substantial differences between catfish and bass in the plasma protein binding and RBC binding affinity of CH_3Hg. The unbound fraction in plasma was more than 14-fold greater in catfish compared to bass (Table 1). The affinity constant of CH_3Hg for bass RBCs (ρ), was over 20 times greater than catfish (Table 1). The pre-injection hcts were similar among the two species and were 23 ± 3 and 26 ± 3% (mean ± SD; n = 4 catfish, n = 5 bass; Table 1) for bass and catfish, respectively. There was little change in the hct during the initial 100 h after injection; however, the hct was approximately 60% of the initial hct for both species by 300 h (data not shown).

DISCUSSION

The results of this study are consistent with previous reports of the slow elimination and long biological half-life of CH_3Hg in fish. Despite the similarities in biological half-life for both species, significant species differences in the V_{ss} and Cl_b estimates were observed (Table 1). The differences in V_{ss} and Cl_b appeared to be explained by the lower plasma protein and RBC binding of CH_3Hg in catfish. For chemicals that are slowly excreted like CH_3Hg, changes in blood and plasma binding will directly affect the clearance with decreased binding increasing the clearance [37]. Similarly, changes in plasma binding can also directly affect the volume of distribution if there is little change in tissue binding [38]. Although catfish used in this study had a higher body lipid content than bass, little correlation between lipid content and CH_3Hg accumulation has been demonstrated [3]. Therefore, the greater V_{ss} in catfish is probably due to decreased plasma binding and/or increased lean tissue binding. The similarity in the biological half-life of CH_3Hg despite significant species differences in Cl_b is due to the larger distributional space in catfish from which CH_3Hg must be cleared.

Mercury has an extremely high affinity for sulfhydryl groups [39] including those of low molecular weight such as glutathione [40,41], and the conventional pharmacological concept of an unbound or free fraction probably does not apply to CH_3Hg. However, CH_3Hg rapidly exchanges between glutathione and hemoglobin in human erythrocytes [40]. Also, the average lifetime of the CH_3Hg–glutathione complex is less than 0.01 s [40,41], suggesting that the pharmacokinetic behavior of CH_3Hg bound to small molecular weight ligands may be analogous to that of an unbound molecule. In our

binding studies, the ultrafiltrate probably represents CH_3Hg bound to small molecular weight ligands that were able to pass through the membrane (30,000 mol. wt. cutoff). A similar result for CH_3Hg has been reported for human plasma, where the ultrafiltrate fraction of CH_3Hg was 0.8 and 8% after filtering plasma through membranes with mol. wt. cutoffs of 1,000 and 300,000 MU [42]. Also, zone electrophoresis of human plasma showed that only 20% of the protein-bound CH_3Hg was associated with albumin and over 45% associated with unidentified proteins of molecular weights greater than albumin [42].

The large difference in blood binding of CH_3Hg between bass and catfish (ρ; Table 1) is similar to the greater binding of CH_3Hg to rat blood cells compared with other mammalian species [18]. The difference in blood cell binding affinity among mammalian species is not due to greater binding to hemoglobin [18] but to some unknown mechanism. Our results indicated that bass were closer to rats in their blood cell affinity for CH_3Hg and catfish are more similar to humans and other mammalian species.

The primary elimination routes of CH_3Hg from rodents and man are biotransformation to inorganic Hg and secretion of CH_3Hg into bile, bound to various nonprotein sulfhydryl compounds [43–45]. Of these two excretory routes, conversion of CH_3Hg to inorganic Hg is considered more important [44]. These elimination pathways also appear to occur in fish, as a significant portion of ^{203}Hg is recovered in the feces after $CH_3{}^{203}Hg$ administration in trout [15], and the isotope ratio of ^{203}Hg to ^{14}C increases over a 6-week period in the kidney and liver of trout coadministered $^{14}CH_3Hg$ and $CH_3{}^{203}Hg$ [46]. The importance of CH_3Hg conversion to inorganic Hg in fish is unclear as most of the Hg in edible fish tissue (>98%) is CH_3Hg [3]. However, after IA administration of inorganic Hg to channel catfish, most of the Hg eventually becomes concentrated in the liver with only trace quantities accumulating in skeletal muscle [47]. Furthermore, the biological half-life of inorganic Hg in channel catfish exceeds 700 d [47], considerably longer than CH_3Hg (34 d, Table 1). These observations indicate that inorganic Hg is much more persistent in fish and the accumulation of inorganic Hg in the liver after CH_3Hg exposure may provide an estimate of CH_3Hg conversion to inorganic Hg.

An additional elimination pathway for CH_3Hg may be branchial. Methyl mercury is lipophilic [48] and rapidly absorbed from water across the gills [13,49]. Assuming the ultrafiltrate or unbound fraction of CH_3Hg in plasma is available to diffuse across the gills, then comparison of the unbound Cl_b (Cl_b/f_u) values relative to gill blood flow (assumed to equal cardiac output) can provide an estimation of the importance of branchial elimination. The cardiac output in channel catfish at 21°C is 2.4 ml/h/g [28]. The cardiac output in largemouth bass has not been reported, but values for other teleosts range from 1.1 to 3.75 ml/h/g [35,36]. The unbound Cl_b for catfish and bass was 0.71 and 2.28 ml/h/g respectively, which is approximately 30% of cardiac output in catfish and probably a higher percentage for bass. This high, unbound Cl_b relative to cardiac output does not rule out biliary excretion and metabolism as important elimination pathways, but it does suggest that branchial excretion of CH_3Hg may be an additional excretory route for CH_3Hg in fish.

The effects of the differences in V_{ss} and Cl_b on the pattern of CH_3Hg elimination is illustrated in Figure 3. This simulation predicts that a greater fraction of an IA dose is initially excreted by bass when the blood concentration is higher than in catfish. However, by the end of the distributional phase (150–250 h), a larger portion of the dose has been excreted by the catfish (Fig. 3). The slopes of the terminal portion of the curves are similar due to the similarity in biological half-lives (Table 1).

Although the model predicts that a greater fraction of an IA-administered dose will be retained by bass (Fig. 3), the body burdens of CH_3Hg in naturally exposed bass and catfish would depend on the exposure conditions and assimilation efficiency from food and water, assuming other factors are equal (i.e., age, body size). Our analysis indicates that under identical exposure conditions, the body burden of CH_3Hg in catfish and bass of similar age and size should be equivalent due to similarities in biological half-life, but that blood and plasma concentrations of CH_3Hg will be higher in bass due to the greater binding to RBCs and plasma proteins.

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