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Toxicokinetics and Disposition of Inorganic Mercury and Cadmium in Channel Catfish after Intravascular Administration

I. R. SCHULTZ,[1] E. L. PETERS, AND M. C. NEWMAN

Savannah River Ecology Laboratory, The University of Georgia, Drawer E, Aiken, South Carolina 29802

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To better understand the distribution and elimination of inorganic mercury (Hg) and cadmium (Cd) in fishes, channel catfish (*Ictalurus punctatus*) were administered either 6.4. μg/kg $^{203}HgCl_2$ or 4.0 μg/kg $^{109}CdCl_2$ via a dorsal aortic cannula. Blood, plasma, and red blood cells (RBCs) were serially sampled up to 156 (Hg) or 335 (Cd) days. The fraction of the injected dose remaining in the animal (X_f) was also determined at selected times by whole animal counting. The blood concentration and X_f–time profiles were simultaneously fitted to a three-compartment toxicokinetic model. The plasma concentration–time profile was also separately fitted to the same three-compartment model for comparison of parameter estimates. Toxicokinetic analysis of the blood concentration and X_f–time profile provided the following values: steady-state volume of distribution = 13.8 ± 2.8 ml/g (Hg), 41.4 ± 0.3 ml/g (Cd); total body clearance = 0.021 ± 0.0006 ml/day/g (Hg), 0.0031 ± 0.0008 ml/day/g (Cd); biological half-life ($t_{\frac{1}{2}}\beta$) = 722 ± 309 days (Hg), 9627 ± 2206 days (Cd). Estimates of the $t_{\frac{1}{2}}\beta$ were up to 94 times longer if determined by simultaneous fitting of the blood concentration and X_f–time profiles. A time-dependent accumulation of Hg and Cd by RBCs was observed with maximum RBC concentrations of Hg and Cd occurring at 7 and 12 days after injection. After injection, the tissues with the highest accumulation of Hg were the liver, trunk and head kidney, muscle, and skin, but the amount of Hg in the liver gradually increased over 156 days. Most of the Cd was accumulated by the liver and trunk kidney within 24 hr, with little change occurring after 335 days. This study demonstrates the usefulness of intravascular injection and simultaneous analysis of blood and whole body amount data in determining the elimination of metals from fishes.

Mercury (Hg) and cadmium (Cd) contamination of aquatic environments is an important ecological and human health concern (Fitzgerald and Clarkson 1991; Nriagu and Pacyna, 1988). Numerous studies have described the high toxicity of these metals to fishes after acute and chronic exposures (reviewed in Mance, 1990). Uptake and accumulation studies of inorganic mercury and cadmium in fishes have described the relatively slow absorption and low bioavailability of these metals from water or food and the importance of both administrative routes in determining the accumulation of these metals (Olson *et al.*, 1973; Williams and Giesy, 1978; Huckabee *et al.*, 1979; Stary *et al.*, 1981; Norrgren *et al.*, 1985; Borg *et al.*, 1988; Harrison and Klaverkamp, 1989; Glynn, 1991). The disposition of inorganic mercury and cadmium in fishes has been characterized after water, oral, and ip administration, with the pattern of tissue distribution varying depending on the administrative route. Regardless of the exposure route, the liver and kidney tended to accumulate the highest quantities of these metals (Weisbart, 1973; Sorenson, 1990).

Depuration or elimination studies of inorganic mercury and cadmium in fishes have relied primarily on water exposure or repetitive oral dosing, followed by whole body estimation of metal content (Pentreath, 1976a,b, 1977; Norrgren and Runn, 1985; Harrison and Klaverkamp, 1989). Reported biological half-life estimates have ranged from 8 to 162 days for inorganic mercury and from 24 to 200 days for cadmium depending on the species, route of administration, and sampling duration (Weisbart, 1973; De Freitas *et al.*, 1974; Pentreath, 1976a, 1977; Harrison and Klaverkamp, 1989). Depuration studies in fishes after water exposure or oral dosing are complicated by the elimination of metal externally adsorbed to the gills or intestinal lumen, which may be eliminated more rapidly than excretion of the absorbed dose (Glynn, 1991). Thus, estimates of the biological half-life after water or oral dosing may be biased downward because a significant portion of the eliminated metal may not have been absorbed internally. Consequently, an improved understanding of the distribution and elimination of mercury and cadmium after intravascular injection would be useful in better characterizing the persistence of these compounds in fishes.

The use of multicompartmental, clearance-volume models and repetitive blood sampling to characterize the toxicoki-

[1] Current address: Battelle PNNL, Molecular Biosciences, P.O. Box 99-P7-53, Richland, WA 99352.

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netics of metals in fishes has rarely been applied. This contrasts with mammalian studies of mercury and cadmium, which have frequently used multicompartmental models to characterize the toxicokinetics of these metals after a variety of administrative routes (Clarkson, 1972; Friberg *et al.*, 1992). Typically, toxicokinetic studies of mercury and cadmium in mammals determine model parameters after characterizing the metal concentration–time profile in blood, plasma, or whole animal (Clarkson, 1972; Thompson and Klaassen, 1983; Gregus and Klaassen, 1986; Nielsen, 1992). A few studies (Garty *et al.*, 1981; Thompson and Klaassen, 1983; Tanaka *et al.*, 1985) have simultaneously characterized the concentration–time profiles in blood and plasma after intravascular administration in rats and have revealed that initially (<1 or 2 hr postinjection) little accumulation of these metals occurs in red blood cells (RBCs). However, the concentration ratios of mercury and especially cadmium in RBCs and plasma determined several days after administration indicated that most of the metal becomes associated with RBCs (Garty *et al.*, 1981; Thompson and Klaassen, 1983; Tanaka *et al.*, 1985). The greater accumulation of these metals in RBCs is an important consideration when comparing toxicokinetic model parameters determined from blood or plasma concentration–time profiles because parameters estimated from the plasma profile may overestimate total body clearance (Cl_b) and produce errors in the estimates of the apparent volume of distribution of a chemical (Gibaldi and Perrier, 1982). Thus, toxicokinetic parameters for mercury and cadmium estimated from blood-referenced models may provide more realistic estimates of the apparent volume of distribution and total body clearance. Because an important aspect in the risk assessment of any contaminant is accurate estimation of the distribution and persistence of the chemical within the organism, it would be of interest to know the differences in the apparent volume of distribution and total body clearance of mercury and cadmium determined from either the plasma or the blood profiles.

We characterized inorganic mercury and cadmium toxicokinetics in channel catfish (*Ictalurus punctatus*) after intravascular administration with a clearance-volume, multicompartmental model. Although methylmercury is the predominant mercury species found in fishes (Bloom, 1993), the inorganic form was used in this study to allow comparison with the similarly divalent cadmium ion. The primary objectives of the study were to simultaneously characterize the blood, plasma, and RBC concentration–time and whole animal amount–time profiles of inorganic mercury and cadmium and to compare the toxicokinetic model parameters estimated using the different profiles. The elimination of both metals was monitored for a longer time period than in previous studies to allow for a more accurate estimation of the biological half-life and total body clearance. The organ distribution of these metals was also determined in a separate group of catfish at selected times after intravascular administration.

METHODS

Fish and Water Quality

Adult channel catfish (160–381 g) of mixed sex were obtained from Orangeburg Aquaculture (Cordova, SC) and maintained in 400-liter recirculating water, fiberglass aquaria (LS 700, Frigid Units, Toledo, OH) containing reconstituted hard water (USEPA, 1978) and 1‰ (w/v) NaCl. The loading density of catfish in the aquaria was maintained below 5 g/liter. Half of the aquarium water was replaced biweekly with aerated, reconstituted water. Chemical characteristics of the freshly prepared water were the following: total alkalinity, 110–120 mg/liter (as $CaCO_3$); hardness, 160–180 mg/liter (as $CaCO_3$) and pH 7.9. Temperature and pH in the aquaria were monitored daily and ranged from 20 to 22°C and 7.7 to 7.9, respectively. Ammonia concentrations in the aquaria were regularly monitored to ensure that the concentration was below 0.5 mg/liter. Catfish were fed a maintenance ration of approximately 2% of their body mass three times per week (Tucker and Robinson, 1990) with soft moist pelleted feed (Rangen Inc., Buhl, ID). Immediately upon arrival, the catfish received a 2-hr treatment in a 0.25 mg/liter solution of malachite green (Sigma Chemical, St. Louis, MO) and were held a minimum of 30 days before use.

Experimental Design

Surgical procedure. Each catfish was fitted with a dorsal aortic cannula using a method similar to that of Kitzman *et al.* (1988) and Stehly and Plakas (1993) with the following modifications: 150 mg/liter MS-222 was used as an anesthetic, 28-G Teflon tubing (Zeus Inc., Raritan, NJ) was used as the cannula material, and an 18-G iv catheter (Angiocath, Becton–Dickinson, Sandy, UT) was used to guide the cannula into the dorsal aorta. The cannulated catfish were placed in floating polyethylene cages (Bain Marie containers, 19-liter, 45-cm diameter), which were perforated to allow water exchange. The cages containing cannulated catfish were placed in oval 568-liter plastic aquaria (Rubbermaid Commercial Products, Inc., Winchester, VA) filled with reconstituted hard water and 1‰ (w/v) NaCl. The end of the cannula was attached to a 1-ml syringe and floated above the fish inside the cage. Catfish were allowed to recover from surgery for a minimum of 24 hr before dosing. A schematic diagram of the experimental design for blood removal and whole animal counting is depicted in Fig. 1.

Dosing and sampling. $^{203}HgCl_2$ and $^{109}CdCl_2$ were obtained from Amersham Inc. (Arlington Heights, IL). Catfish were injected intra-aortically with either $^{203}HgCl_2$ (6.4 μg/kg total Hg; 222 kBq/kg) or $^{109}CdCl_2$ (4.0 μg/kg total Cd; 847 kBq/kg) dissolved in a modified Cortland saline [as described by Houston *et al.* (1990) except that albumin was not added]. Two protocols were used sequentially to obtain blood samples and whole animal activity estimates. Initially, serial blood samples were removed via the cannula at 0.25, 0.5, 0.75, 1, 2, 3, 6, 12, 24, 36, 48, 72, 120, and 196 hr after injection. The catfish were not fed during this initial 196-hr sampling period. After the blood sample had been removed at 24 hr, the whole animal activity of ^{203}Hg or ^{109}Cd was quantified by placing the unanesthetized catfish for 3 min in a 4-liter Marinelli beaker (GA-MA & Associates, Inc., Miami, FL) containing 1 liter of water. The catfish were returned to their cage in the aquarium immediately after counting. At 120 hr, the catfish were transferred to a constantly aerated 15-liter solution of MS-222 (150 mg/liter) for 2 min (sufficient time for stage II anesthesia to develop) and then placed in the Marinelli beaker for counting. The catfish were not anesthetized within 24 hr of injection to avoid potential anesthetic interference during the initial rapid decline phase in the concentration of the metals in blood and plasma.

After 196 hr, the cannula was removed while the catfish were anesthetized, and later blood samples were obtained from the dorsal aorta of the

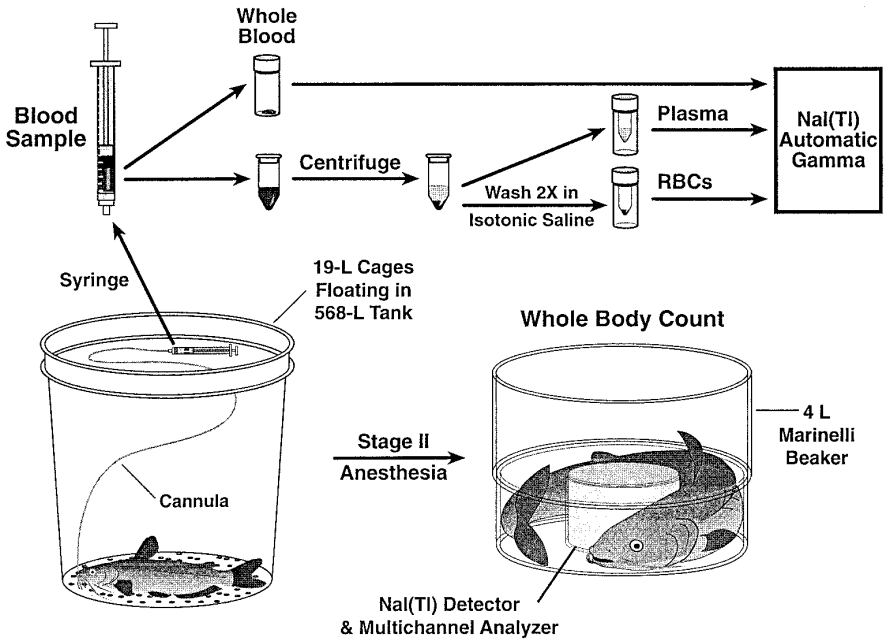

FIG. 1. Diagrammatic representation of the experimental protocol used to collect blood, plasma, red blood cell (RBC) samples, and whole animal measurements. Channel catfish were held in 19-liter cages and administered either $^{203}HgCl_2$ or $^{109}CdCl_2$ via a dorsal aortic cannula and serial blood samples were removed up to 155 days (Hg) or 335 days (Cd) after administration. For each blood sample removed, an aliquot of whole blood was counted separately and the remaining sample was centrifuged and the plasma removed and counted. The RBCs were washed twice in 10 vol of 0.9% NaCl and counted. At periodic intervals the catfish were lightly anesthetized and placed in a 4-liter Marinelli beaker and whole animal activities determined using a NaI(T1) detector.

anesthetized catfish using a 25-G needle and a 1-ml syringe immediately before whole animal counting. After 196 hr, the sample interval for blood removal and whole animal counting was at least 7 days and increased gradually because minimal change in the blood concentration and whole animal activities of the metals was occurring. Sampling continued for 156 days for Hg and 335 days for Cd. Periodically, anesthetized catfish were weighed before placement in the Marinelli beaker. After 196 hr, catfish were fed approximately 2% of their body mass 3 days per week and later reduced to twice weekly, which was sufficient for caged catfish to maintain a body weight within 10% of the initial body weight. After resumption of feeding, samples were collected at least 24 hr after the last feeding period.

Aliquots of whole blood, plasma, and RBCs were separated from each blood sample. The sampling protocol used to obtain blood, plasma, RBC, and whole body activities is depicted in Fig. 1. After removal of a blood sample, a fraction was reserved for whole blood determination, and the remainder was centrifuged for 5 min at 2000*g* to obtain plasma. The RBC pellet was washed twice by resuspension in a 10-fold excess of 0.9% (w/v) NaCl and then centrifuged again, discarding the supernatant after each wash. The sample volumes were quantified gravimetrically assuming a density of 1.05 for whole blood and 1.0 for plasma (Holmes and Donaldson, 1969). The total quantity of blood removed during the initial 196 hr was less than 10% of the estimated blood volume (assumed to be 4% of body weight). After 196 hr, no more than 3% of the estimated blood volume was removed for each sample.

Gamma counting and determination of Hg and Cd. The ^{203}Hg and ^{109}Cd activities of blood, plasma, and RBCs were determined by counting the samples to a 2 σ error of 1% in an automated 7.6-cm wide $\times$ 8.3-cm high well-type NaI(T1) gamma counter (Packard Auto-gamma Model 5530, Packard Instrument Company, Meriden, CT). Counts were corrected for radioactive decay to a standard reference date and converted to values of stable Hg or Cd using specific activities calculated from ^{203}Hg and ^{109}Cd standards. The ^{203}Hg or ^{109}Cd activity of the whole fish was estimated using a 7.6-cm wide $\times$ 7.6-cm high well-type NaI(T1) solid scintillator detector/photomultiplier and multichannel analyzer (Canberra Series 85, Canberra, Meriden, CT). The Marinelli beaker containing the catfish was placed over the detector (Fig. 1) and the gamma emissions were counted for 3 min. Background count rates were determined using a water-filled Marinelli beaker in the same geometry used with catfish. The net count rates were corrected for radioactive decay to the same reference date as that for the blood products. At completion of the study, the catfish were killed by anesthetic overdose, placed in the Marinelli beaker, and the whole body count rate was estimated from three separate measurements of 5 min each. Immediately afterwards, the catfish were homogenized in a Waring blender and aliquots of the homogenate counted in the Packard NaI(T1) gamma counter. The whole body count rate was standardized to the Packard gamma counter using the count rate ratio of the whole body count rate to the count rate for the homogenate. The fraction of the injected dose remaining in the animal (X_f) was calculated as the ratio of the adjusted whole body count rate divided by the activity of the dose that was determined using the Packard gamma counter.

Hematocrit adjustment. The hematocrit (hct) in nonanesthetized catfish was assumed to be 17%. This value is based on unpublished observations in our laboratory of hct values from cannulated catfish (17.0 $\pm$ 5%; n = 10 $\pm$ SD) and is similar to values reported by other researchers (McKim *et al.,* 1994; Bai and Gatlin, 1994). Reports of higher hct values obtained from channel catfish previously anesthetized or stunned (reviewed in McKim *et al.,* 1994) indicated that the blood sampling protocol influences the hct. Several explanations have been proposed for this phenomenon (Houston *et al.,* 1969; McKim *et al.,* 1994), but we assumed that hct determined via the cannula in nonanesthetized catfish is a closer approximation of the hct in undisturbed catfish. In our study, the hct of blood samples obtained under anesthesia was measured using heparinized microhematocrit

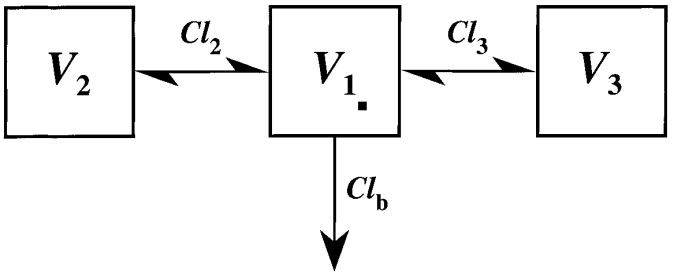

FIG. 2. Conceptual model of Hg and Cd toxicokinetics in channel catfish: V_1, V_2, and V_3 are apparent volumes of distribution of the central and peripheral compartments; Cl_2 and Cl_3 are intercompartmental clearances and Cl_b is total body clearance.

tubes, and Hg or Cd concentrations in whole blood and washed RBCs were adjusted to a hct value of 17% by the following equation:

$$C_b = C_0 - ((\text{hct} - 0.17) \cdot C_{RBC}) + ((\text{hct} - 0.17) \cdot C_p), \quad (1)$$

where C_b is the adjusted blood concentration (ng/ml), C_0 is the observed blood concentration (ng/ml), C_{RBC} is the washed RBC concentration (ng/ml), and C_p is the plasma concentration (ng/ml). The washed RBC concentration was calculated by the following equation:

$$C_{RBC} = X_{RBC}/(\text{blood volume} \cdot \text{hct}), \quad (2)$$

where X_{RBC} is the amount of Hg or Cd in the washed RBCs (ng), and blood volume was the volume of the blood sample used to obtain the washed RBCs.

Distribution. Channel catfish weighing 130 ± 30 g (mean ± SD; n = 20) were administered $^{203}HgCl_2$ or $^{109}CdCl_2$ with the same dose and procedures described previously. The catfish were terminated at 1, 8, 30, and 118 days (Hg only) after injection, and the following tissues and fluids were removed: blood, plasma, liver, bile, stomach, intestines, head and trunk kidneys, swim bladder, gonads, heart, spleen, abdominal fat, skin, white and red muscle, and brain. All tissues except muscle, skin, and blood were completely removed. Several 2- to 5-g aliquots of white muscle and a single 1- to 2-g red muscle sample was removed. The relative masses of these tissues in catfish were assumed to be similar to those of rainbow trout (*Oncorhynchus mykiss*), i.e., 55 and 5% of the body weight as white and red muscle, respectively (Giblin and Massaro, 1973; Weatherly and Gill, 1987). Skin was removed from one side of the catfish, trimmed of any attached muscle, and the activity adjusted for the whole tissue assuming 3% of body weight was skin (unpublished data). The ^{203}Hg or ^{109}Cd activity in all tissue samples was determined using the Packard gamma counter. The catfish used in the toxicokinetic portion of the study were also dissected and these tissues plus gill filaments were counted prior to homogenization with the rest of the carcass.

Toxicokinetic analysis. A preliminary analysis was performed comparing several two- and three-compartment models with elimination from the central compartment or from both the central and a peripheral compartment. We concluded that a three-compartment model with elimination only from the central compartment provided the best fit of the whole animal fraction, blood, and plasma concentration–time profiles for Hg and Cd. This three-compartment clearance-volume model is shown in Fig. 2. This conclusion was based on comparison of several criteria: Akaike information criterion, coefficients of variation, and visual inspection of the fits (Boxenbaum *et al.*, 1974). The following model-based equations were simultaneously fitted to the experimentally determined blood concentration–time profile and dose fraction remaining–time profile for Hg and Cd using NONLIN (Metzler, 1974), a nonlinear least-squares computer program:

$$C_b = Ae^{-\pi t} + Be^{-\alpha t} + Ce^{-\beta t} \quad (3)$$

$$X_f = \frac{(A/\pi)e^{-\pi t} + (B/\alpha)e^{-\alpha t} + (C/\beta)e^{-\beta t}}{A/\pi + B/\alpha + C/\beta}, \quad (4)$$

where X_f is the the fraction of the dose remaining in the animal at any time (t). Equation (4) is based on Chiou (1972) and assumes that elimination occurs only from the central compartment. The plasma concentration–time profile [$C_p(t)$] was fitted independently to Eq. (3). A Y^{-2} weighting function was used for the blood and plasma concentration–time profiles and a Y = 1 weight was used for the fraction of the dose remaining–time profile [$X_f(t)$]. The derived model parameters were total body clearance (Cl_b), intercompartmental clearances between the central and peripheral compartments (Cl_2, Cl_3), and the apparent volumes of distribution (V_1, V_2, V_3). The apparent volumes of the peripheral compartments were determined after calculation of the central compartment volume and intercompartmental transfer rate constants as described in Gibaldi and Perrier (1983). The clearance constants were determined directly by substituting Cl_i/V_i for the usual rate constants. Initial estimates of A, B, C, π, α, and β were made using the method of residuals (Gibaldi and Perrier, 1983). The area under the concentration vs time curve (AUC), biological half-life ($t_{\frac{1}{2}}$, β), steady-state volume of distribution (V_{ss}), and mean residence time (MRT) were estimated using the following equations: AUC = $A/\pi + B/\alpha + C/\beta$, $t_{\frac{1}{2}}$, β, = $0.693/\beta$, V_{ss} = $V_1 + V_2 + V_3$, and MRT = AUMC/AUC [AUMC is the area under the moment curve calculated as dose $\cdot (A/\pi^2 + B/\alpha^2 + C/\beta^2)$].

RESULTS

Toxicokinetic Analysis of Hg

The concentration of Hg in blood and plasma declined rapidly in the first 12 hr after intra-arterial injection and then decreased slowly, with measurable ^{203}Hg activity still present after 155 days (Figs. 3 and 4). The concentration of Hg in washed RBCs initially declined in the first 12 hr before increasing to a maximum concentration at 7 days, and decreased thereafter (Fig. 4). The decline in the ^{203}Hg activity of the whole animal was also slow with an average of 48% of the dose excreted by 155 days (Fig. 3, inset). A three-compartment toxicokinetic model adequately described both the $C_b(t)$ and the $X_f(t)$ profiles when fitted simultaneously (Fig. 3). This model also adequately described the $C_p(t)$ profile in a separate fit (Fig. 4). The parameter values from the nonlinear least-squares fitting of the $C_b(t)$ and $X_f(t)$ profiles and the $C_p(t)$ profile are shown in Table 1.

Toxicokinetic analyses indicated that Hg distributed into three distinct compartments within the catfish: a small central compartment (V_1) that approximated the blood or plasma volume of the catfish, a larger peripheral compartment (V_2), and a very large peripheral compartment (V_3) that accounted for up to 94% of the V_{ss} (Table 1). The intercompartmental clearances (Cl_2, Cl_3) between the central and peripheral compartments were small, indicating slow exchange of Hg between these compartments (Table 1). Several parameters were substantially different after the simultaneous fitting of

the $C_b(t)$ and the $X_f(t)$ profiles compared with the estimates derived from the $C_p(t)$ profile (Table 1). The apparent volumes of the peripheral compartments (V_2, V_3) were as much as 8-fold higher and the intercompartmental clearances (Cl_2, Cl_3) were 15- to 248-fold lower if estimated simultaneously from the $C_b(t)$ and $X_f(t)$ profiles (Table 1). The biological half-life ($t_{\frac{1}{2}}^{\beta}$) was more than 15 times longer than the value estimated from the $C_p(t)$ data despite modest differences in the total body clearance (Cl_b) (Table 1). In general, the major differences in parameter estimates determined from the C_p or $C_b + X_f(t)$ profiles were progressively larger peripheral storage compartments, smaller intercompartmental clearances, and increased biological half-life (Table 1).

Disposition of Hg

All tissues examined had detectable amounts of Hg after 24 hr. The liver, trunk and head kidneys, white and red muscle, and skin accumulated most of the injected Hg (Table 2). For many tissues, the amount of Hg reached a maximum value by 8 days and then declined slowly (Table 2). A significant exception was the liver, which continued to accumulate Hg throughout the experiment and contained more than 35% of the injected Hg after 156 days (Table 2). Only small quantities of Hg were detected in bile. The tissue/blood concentration ratios increased throughout the exposure for most tissues and a steady-state concentration ratio had not been achieved after 156 days (Table 3). By the end of the experiment, tissue/blood concentration ratios in all tissues were greater than one, but the liver and trunk and head kidneys had exceptionally large ratios, suggesting that some form of sequestration was occurring in these tissues (Table 3).

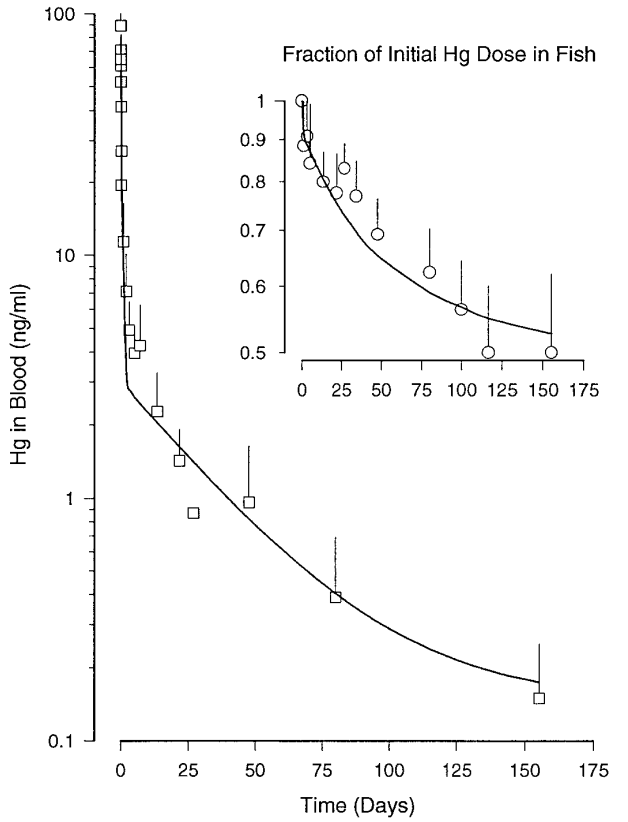

FIG. 3. Hg blood (□) concentration–time profile and the fraction of the injected dose (○) remaining in the fish (X_f, t) profile (inset figure) after intra-arterial injection of 6.4 μg/kg Hg as $HgCl_2$. Symbols represent experimentally determined values (mean ± SD, n = 6–8) and the lines show the simultaneous least squares fit to Eqs. (3) and (4). Error bars not shown fit within the data point.

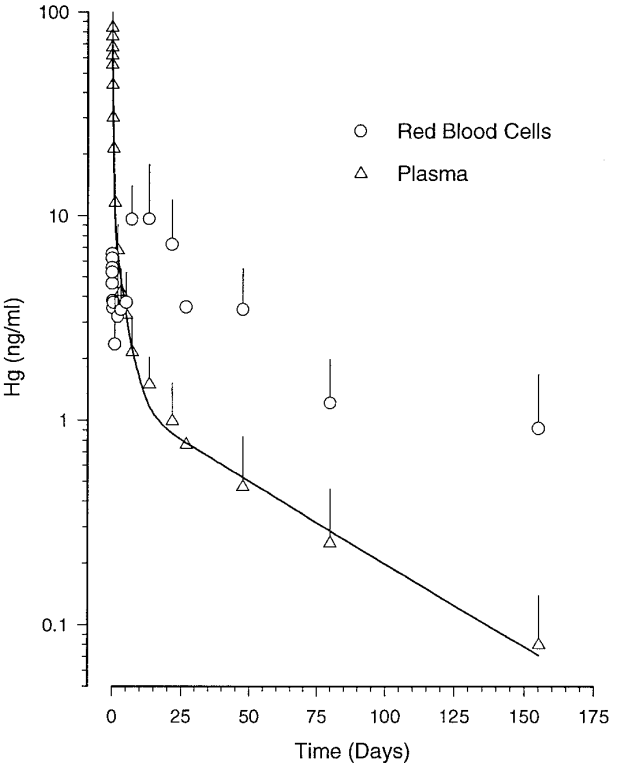

FIG. 4. Hg plasma (△) and red blood cell (○) concentration–time profile after intra-arterial injection of 6.4 μg/kg Hg as $HgCl_2$. Symbols represent experimentally determined values (mean ± SD, n = 6–8) and the line is the least squares fit to Eq. (3). Error bars not shown fit within the data point.

Toxicokinetic Analysis of Cd

The concentrations of Cd in blood and plasma rapidly declined within the first 24 hr after injection and then decreased slowly (Figs. 5 and 6), with plasma concentrations approaching a constant value by 22 days (Fig. 6). The concentration of Cd in washed RBCs had a similar profile to that observed for Hg—an initial decrease followed by an increase to a maximum value at 12 days, and a subsequent log-linear decline (Fig. 6). The decline in the activity of ^{109}Cd in the whole animal was extremely slow, with an average of only 8% of the injected dose

TABLE 1
Toxicokinetic Parameters for ^{203}Hg and ^{109}Cd in Channel Catfish after Intravascular Administration of 6.4 ng/g ^{203}Hg as $HgCl_2$ and 4.0 ng/g ^{109}Cd and $CdCl_2$

	^{203}Hg		^{109}Cd	
	Concentration–time profile[a,b]		Concentration–time profile[a,b]	
Parameter	Blood + X_f	Plasma	Blood + X_f	Plasma
V_1 (ml/g)	0.108 ± 0.016	0.090 ± .006	0.059 ± .0087	0.044 ± .006
V_2 (ml/g)	1.276 ± .210	0.209 ± .014	2.491 ± .0144	0.112 ± .026
V_3 (ml/g)	12.39 ± 2.59	1.467 ± .650	38.87 ± .273	15.48 ± 1.96
V_{ss} (ml/g)	13.77 ± 2.82	1.557 ± .670	41.42 ± .30	15.63 ± 1.99
Cl_2 (ml/day/g)	0.015 ± .005	0.161 ± .0026	0.172 ± .0213	0.090 ± .622
Cl_3 (ml/day/g)	0.00025 ± .0001	0.062 ± .0006	0.0544 ± .013	0.057 ± .002
Cl_b (ml/day/g)	0.021 ± .0006	0.055 ± .0021	0.0031 ± .0008	0.120 ± .010
AUC (ng/ml day)	299.3 ± 4.9	117.0 ± 4.4	1,288 ± 25	33.66 ± 2.9
$t_{\frac{1}{2}}$, β (days)	722.5 ± 309	37.2 ± 2.8	9,627 ± 2206	278 ± 87
MRT (days)	644.6 ± 59	32.3 ± 2.3	13,215 ± 3000	130 ± 59

Note. Parameter values are from a NONLIN fit of model-based equations to data shown in Figs. 3 and 4.

[a] The blood concentration–time profile was simultaneously fitted with the fraction remaining (X_f)–time profile to obtain estimates of A, B, C, π, α, and β that were used to calculate the model parameters. The plasma concentration–time profile was fitted separately to Eq. (3) and model parameters based on this additional fit are presented for comparison.

[b] ± SD. Parameter estimates ± SD were calculated by the NONLIN program (^{203}Hg: n = 8 catfish, mean body weight ± SD = 250 ± 63 g; ^{109}Cd: n = 7, mean body weight ± SD = 179.0 ± 21 g).

excreted by 335 days (Fig. 5, inset). The three-compartment toxicokinetic model used to describe the toxicokinetics of Hg was also adequate to describe the C_p or $C_b + X_f(t)$ profiles of Cd (Figs. 5 and 6). The parameter values determined after the nonlinear least-squares fitting of the various concentration–time profiles are shown in Table 1.

TABLE 2
Whole Organ Distribution (Percentage of Injected Dose) of ^{203}Hg in Channel Catfish after Intravascular Administration of 6.4 ng/g ^{203}Hg as $HgCl_2$

	Days after administration[a]				
Tissue	1	8	30	118	156
Liver	11.6 ± 1.7	15.4 ± 0.8	19.6 ± 3.5	28.6 ± 3.4	35.9 ± 4.7
Trunk kidney	14.6 ± 2.6	18.7 ± 2.3	17.8 ± 4.4	12.2 ± 3.7	9.32 ± 2.9
Head kidney	4.38 ± 2.4	5.84 ± 1.6	2.66 ± 0.3	2.41 ± 0.2	1.61 ± 0.5
Stomach	0.70 ± 0.1	0.49 ± 0.08	0.53 ± 0.1	0.46 ± 0.01	1.34 ± 1.5
Intestine	1.95 ± 0.5	1.55 ± 0.1	1.91 ± 0.1	0.85 ± 0.03	1.02 ± 0.2
Gonad	1.25 ± 1.2	2.88 ± 3.3	3.57 ± 1.6	1.73 ± 1.4	1.03 ± 0.6
Spleen	1.02 ± 0.3	1.31 ± 0.2	0.90 ± 0.2	0.80 ± 0.06	0.39 ± 0.1
Red muscle[b]	7.01 ± 2.2	7.30 ± 3.9	5.89 ± 3.1	1.81 ± 0.7	3.75 ± 2.1
Skin[b]	3.48 ± 0.05	3.21 ± 0.4	1.80 ± 0.5	0.69 ± 0.1	0.75 ± 0.4
Swim bladder	0.40 ± 0.1	0.21 ± 0.01	0.20 ± 0.01	0.27 ± 0.01	0.46 ± 0.4
White muscle[b]	13.6 ± 6.9	21.3 ± 7.0	11.8 ± 3.4	9.35 ± 4.6	7.90 ± 2.7
Fat	0.29 ± 0.06	0.38 ± 0.06	0.14 ± 0.02	0.13 ± 0.06	0.14 ± 0.09
Heart	0.18 ± 0.05	0.13 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.12 ± 0.03
Brain	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	n.d.	0.09 ± 0.05
Bile	0.04 ± 0.002	0.10 ± 0.04	0.22 ± 0.09	n.d.	0.04 ± 0.02
Gill fillaments	n.d.	n.d.	n.d.	n.d.	0.05 ± 0.02
Blood[c]	4.07 ± 4.7	2.08 ± 0.5	1.66 ± 0.4	n.d.	0.18 ± 0.09

[a] Mean ± SD of 3–5 catfish except 118 days, n = 2. n.d., not determined.

[b] White and red muscle and skin was estimated as 55, 5, and 3% of body weight, respectively.

[c] Blood volume was estimated as 4% of body weight.

TABLE 3
Tissue to Blood Concentration Ratios for ^{203}Hg after Intravascular Administration of 6.4 ng/g ^{203}Hg as $HgCl_2$

	Days after administration[a]				
Tissue	1	8	30	118	156
Liver	6.4 ± 3	24 ± 3	65 ± 27	370 ± 27	990 ± 470
Trunk kidney	16 ± 7	53 ± 8	62 ± 17	270 ± 130	590 ± 300
Head kidney	13 ± 6	47 ± 11	37 ± 13	160 ± 40	360 ± 190
Stomach	0.4 ± 0.1	1.1 ± 0.2	1.6 ± 0.5	8.1 ± 2	30 ± 28
Intestine	0.9 ± 0.3	0.7 ± 0.3	6.1 ± 2	18 ± 3	27 ± 13
Gonad	0.6 ± 0.3	2.9 ± 1	9.5 ± 6	56 ± 42	24 ± 11
Spleen	7.7 ± 4	22 ± 6	25 ± 8	170 ± 44	170 ± 110
Red muscle	0.7 ± 0.1	2.5 ± 1	2.7 ± 0.9	7.6 ± 4	23 ± 17
Skin	0.6 ± 0.2	2.0 ± 0.2	1.4 ± 0.2	4.8 ± 0.1	6.9 ± 2
Swim bladder	0.6 ± 0.2	1.1 ± 0.3	1.4 ± 0.4	7.0 ± 2	13 ± 7
White muscle	0.2 ± 0.1	0.7 ± 0.3	0.5 ± 0.2	3.4 ± 2	5.6 ± 4
Fat	0.3 ± 0.2	0.3 ± 0.2	1.6 ± 0.6	2.6 ± 1	4.0 ± 1
Heart	0.7 ± 0.2	2.7 ± 0.7	3.9 ± 0.2	13 ± 2	35 ± 17
Brain	0.05 ± 0.02	0.3 ± 0.08	0.5 ± 0.1	n.d.	22 ± 20
Bile	0.2 ± 0.1	2.0 ± 0.5	3.4 ± 2.2	n.d.	7.6 ± 4
Gill filaments	n.d.	n.d.	n.d.	n.d.	4.9 ± 2

[a] Mean ± SD of 3–5 catfish except 118 days, $n = 2$. n.d., not determined.

Toxicokinetic analyses for Cd indicated the same general description given for Hg, except that Cd had an even larger peripheral compartment (V_3), which comprised 97% of the steady-state volume of distribution (Table 1). A 38-fold lower estimate of Cl_b was obtained using the $C_p(t)$ profile compared with the $C_b + X_f(t)$ profile. The biological $t_{\frac{1}{2}}$, $g\beta$ for Cd estimated from the $C_b + X_f(t)$ profiles was over 9000 days (Table 1) and reflected the large distributive volume and the negligible capacity of channel catfish to excrete Cd.

Disposition of ^{109}Cd

The highest accumulations of Cd were observed in the liver and trunk kidney, with lesser amounts in white muscle, red muscle, and skin (Table 4). The remaining tissues accumulated only small quantities of Cd, typically less than 2% of the injected dose (Table 4). The pattern of tissue distribution appeared to be established within 24 hr with little change observed over the next 334 days (Table 4; Fig. 7). The tissue/blood concentration ratios (Table 5) showed a trend similar to that observed for Hg, i.e., the ratios increased throughout the experiment. At 335 days, the ratios for the liver and trunk kidney were more than 2000, a further indication of the extremely high affinity of Cd for these tissues (Table 4).

DISCUSSION

The large V_{ss}'s determined for inorganic Hg and Cd reflected the extensive binding of these metals in peripheral tissues compared with blood or plasma. Fish are similar to mammals in possessing a number of high-affinity metal binding proteins such as the metallothioneins (MT) (Roesijadi, 1992), and the large V_{ss}'s were probably an indication of the high capacity of these binding proteins to sequester Hg and Cd from blood or plasma.

Both Hg and Cd have higher affinities for RBCs than for plasma, as shown by the high RBC/plasma concentration ratio during the terminal portion of the concentration–time profiles (Figs. 4, 6, and 8). Analyzing the $C_b(t)$ profile simultaneously with the $X_f(t)$ profile increased the estimates of V_{ss} and decreased the estimates of Cl_b for both Hg and Cd, resulting in increases in the biological half-life estimates up to 94-fold (Table 1). Although 48% of the injected Hg was excreted by 156 days, the biological half-life estimate was actually 722 days due to the multiexponential pattern of elimination predicted by the model. Thus, a greater proportion of the dose of Hg was excreted initially when blood levels and most tissue concentrations were higher. A similar pattern has been observed in rats after iv injection of inorganic Hg, where the whole body elimination curve was observed to be triexponential and the half-life of the terminal phase was reported to be 90–100 days (Rothstein and Hayes, 1960).

The terminal blood concentrations of both Hg and Cd appeared to decline in a log-linear manner after the initial distributive phases were complete (Figs. 3 and 5). This behavior would normally be interpreted as the result of excretion of the metals from the animal. However, it is clear from the $X_f(t)$ profiles (Figs. 3 and 5, insets) that elimination of

the metals was slower than would be predicted from the terminal decline of the $C_b(t)$ and $C_p(t)$ profiles only. The intercompartmental clearance between the central and deep compartments (Cl_3) was over 80 times smaller than the total body clearance for Hg (Table 1, blood + X_f). This suggests that a disequilibrium between Hg in blood and peripheral tissues may have existed, with the elimination of Hg from the whole animal influenced more by the slow transfer of Hg from storage tissues to the blood. This would result in a more rapid loss of Hg from blood than from peripheral storage tissues. A similar explanation for the continued decline in blood concentrations of Cd is less clear because only negligible quantities of Cd were excreted by the catfish during the study, and the Cl_3 is much larger than Cl_b (Table 1, blood + X_f). The rapid initial distributive phase for Cd appears to have established the principal organ distribution pattern by 24 hr, with <3% of the dose remaining in the blood (Table 4). The gradual loss from blood of this small fraction of the total dose may be due to slow, continued transfer to peripheral tissues in addition to excretion.

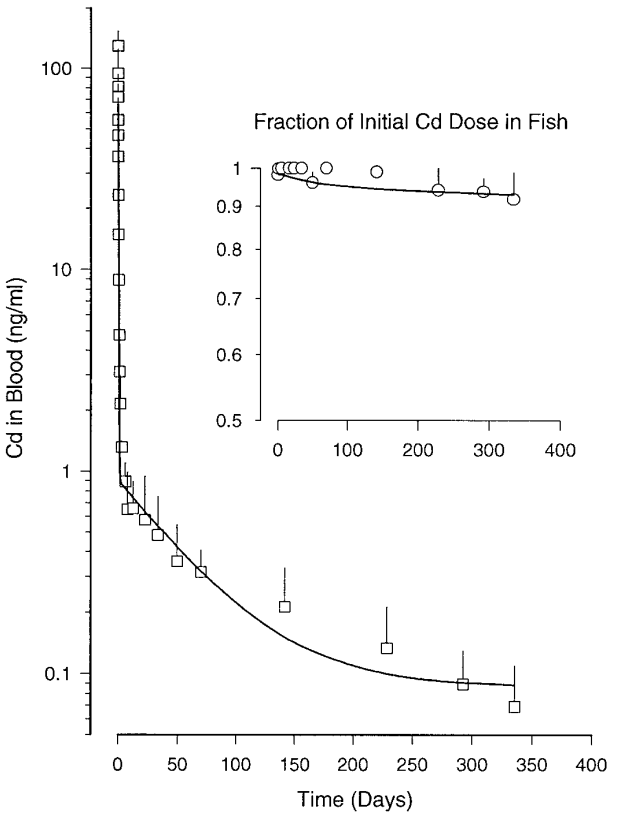

FIG. 5. Cd blood (□) concentration–time profile and the fraction of the injected dose (○) remaining in the fish (X_f, t) profile (inset figure) after intra-arterial injection of 4.0 μg/kg Cd as $CdCl_2$. Symbols represent experimentally determined values (mean ± SD, n = 5–7) and the lines show the simultaneous least squares fit to Eqs. (3) and (4). Error bars not shown fit within the data point.

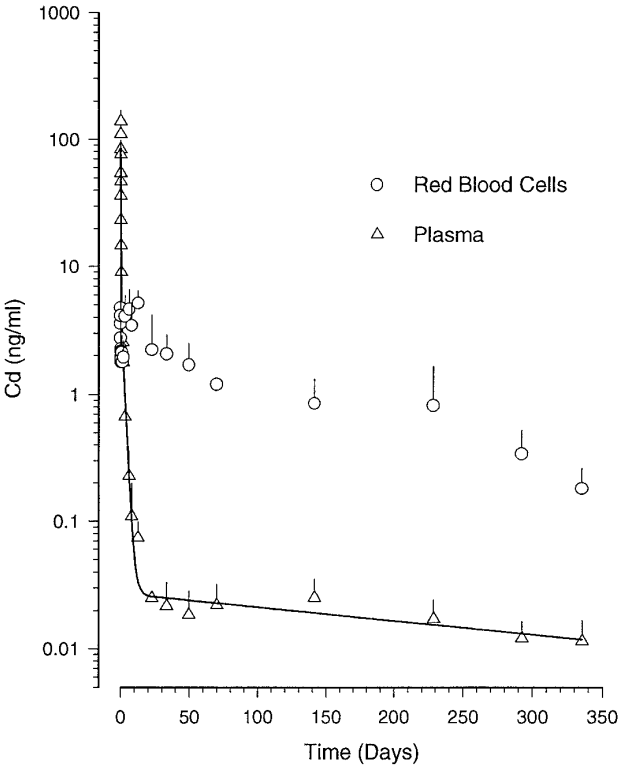

FIG. 6. Cd plasma (△) and red blood cell (○) concentration–time profile after intra-arterial injection of 4.0 μg/kg Cd as $CdCl_2$. Symbols represent experimentally determined values (mean ± SD, n = 6–8) and the line is the least squares fit to Eq. (3). Error bars not shown fit within the data point.

An additional factor influencing the decline of Cd in blood was the apparent redistribution of Cd from plasma to RBCs (Figs. 6 and 8). A similar phenomenon has been described in mammals in which Cd concentrations in RBCs after iv injection reach a maximum value between 2.5 and 5 days (Nordberg *et al.*, 1971; Garty *et al.*, 1981; Tanaka *et al.*, 1985). The maximum RBC concentration of Cd in our study was reached at 12 days when the RBC/plasma concentration ratio exceeded 90 (Fig. 8). This ratio gradually decreased after 12 days and was less than 16 after 335 days, indicating that Cd was being eliminated faster from RBCs than from plasma (Figs. 6 and 8).

A redistribution of Hg to RBCs also appeared to occur after injection. The highest concentration of Hg in RBCs was observed 7 days after administration (Fig. 4). This was similar to the pattern observed for Cd, except the RBC/plasma ratio for Hg was lower (Fig. 8). The accumulation of Hg by RBCs observed in our study contrasts with an *in vitro* study of rainbow trout blood (Olson *et al.*, 1973), which found little accumulation by RBCs after a 24-hr incubation with inorganic Hg.

The V_{ss} of Hg was less than one-third of that for Cd, and

TABLE 4
Whole Organ Distribution (Percentage of Injected Dose) of ^{109}Cd in Channel Catfish after Intravascular Administration of 4.0 ng/g ^{109}Cd as $CdCl_2$

Tissue	Days after administration[a]			
	1	8	30	335
Liver	31.4 ± 4.2	26.0 ± 4.5	27.3 ± 6.4	27.9 ± 3.1
Trunk kidney	30.0 ± 3.6	32.0 ± 8.0	35.2 ± 11	36.7 ± 7.2
Head kidney	2.04 ± 0.6	2.19 ± 0.9	3.10 ± 1.2	2.63 ± 1.1
Stomach	0.88 ± 0.1	0.79 ± 0.1	0.86 ± 0.05	0.73 ± 0.2
Intestine	2.03 ± 0.2	1.73 ± 0.4	1.86 ± 0.3	0.99 ± 0.2
Gonad	1.20 ± 0.6	0.59 ± 0.2	1.84 ± 0.2	0.58 ± 0.3
Spleen	0.59 ± 0.2	1.01 ± 0.4	1.04 ± 0.5	0.69 ± 0.4
Red muscle[b]	2.08 ± 0.5	2.77 ± 1.9	3.61 ± 1.1	3.40 ± 1.4
Skin[b]	2.94 ± 0.8	2.81 ± 0.2	1.98 ± 0.6	1.70 ± 0.3
Swim bladder	0.45 ± 0.2	0.32 ± 0.1	0.24 ± 0.03	0.17 ± 0.03
White muscle	5.34 ± 1.5	5.09 ± 1.0	4.32 ± 1.7	4.92 ± 2.0
Fat	0.27 ± 0.04	0.22 ± 0.04	0.48 ± 0.001	0.25 ± 0.2
Heart	0.73 ± 0.5	0.44 ± 0.07	0.34 ± 0.04	0.24 ± 0.09
Brain	0.04 ± 0.004	0.04 ± 0.004	0.05 ± 0.002	0.04 ± 0.02
Bile	0.003 ± 0.002	0.007 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Gill filaments	n.d.	n.d.	n.d.	0.22 ± 0.09
Blood[c]	2.32 ± 0.6	1.05 ± 0.39	1.03 ± 0.58	0.07 ± 0.02

[a] Mean ± SD of 3–5 catfish. n.d., not determined.
[b] White and red muscle and skin was estimated at 55, 5, and 3% of body weight.
[c] Blood volume was estimated as 4% of body weight.

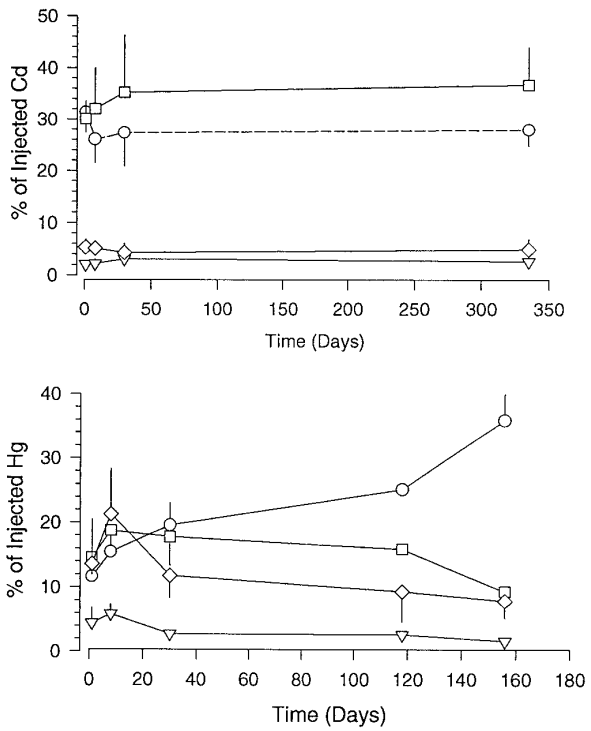

FIG. 7. The percentage of the injected dose accumulated by the liver (○), trunk kidney (□), head kidney (◇), and white muscle (▽) after intra-arterial injection of either 6.4 μg/kg Hg as $HgCl_2$ or 4.0 μg/kg Cd as $CdCl_2$. Mean ± SD, n = 2–5; error bars not shown fit within the data point.

the Cl_b was considerably greater if estimated from the C_b and $X_f(t)$ profiles (Table 1). This indicated that catfish had a greater capacity to excrete Hg relative to Cd, but a lesser ability to sequester or accumulate Hg in peripheral tissues. The differences in elimination between inorganic Hg and Cd were consistent with previously published studies in fishes (Weisbart, 1973; Pentreath, 1977; Harrison and Klaverkamp, 1989; Sorenson, 1991) and are generally considered to be a consequence of the greater retention of Cd in the liver and kidney compared to Hg (Sorensen, 1991). Comparison of the liver and kidney/blood concentration ratios for Hg and Cd (Tables 3 and 5) supported this conclusion because this ratio was always greater for Cd.

The initial pattern of Hg disposition in catfish was similar to that of Cd, with the majority of the dose concentrated in the liver, trunk kidney, and white and red muscle (Table 1). However, the amount of Hg in the liver increased over time despite elimination of Hg from other tissues, and by the end of the study the majority of Hg in the catfish was in the liver (Table 3; Fig. 7). This final pattern of disposition for Hg in catfish was different than for mammals, in which the greatest quantity of Hg accumulates in the kidney (Clarkson, 1972; Gregus and Klaassen, 1986). The time-dependent increase of Hg in the liver suggested that a redistribution of Hg from other tissues to the liver had occurred. This contrasted with the dispositional pattern of inorganic Hg and Cd in rats after iv injection, in which a time-dependent redistribution to the kidney occurred

TABLE 5

Tissue to Blood Concentration Ratios for ^{109}Cd after Intravascular Administration of 4.0 ng/g ^{109}Cd as $CdCl_2$

Tissue	Days after administration[a]			
	1	8	30	335
Liver	43 ± 5	125 ± 35	200 ± 130	2150 ± 850
Trunk kidney	102 ± 21	192 ± 46	298 ± 140	6480 ± 2300
Head kidney	25 ± 14	63 ± 41	66 ± 6	113 ± 410
Stomach	1.6 ± 0.1	3.7 ± 1	4.8 ± 3	58 ± 26
Intestine	2.9 ± 0.3	6.6 ± 2	10 ± 6	57 ± 28
Gonad	1.8 ± 0.4	9.1 ± 4	8.2 ± 3	139 ± 130
Spleen	14 ± 4	61 ± 35	47 ± 19	666 ± 500
Red muscle	0.70 ± 0.1	3.4 ± 2.8	3.9 ± 2	59 ± 30
Skin	1.7 ± 0.5	4.2 ± 1.6	3.5 ± 2	30 ± 11
Swim bladder	1.7 ± 0.7	3.2 ± 1.5	3.6 ± 1	35 ± 17
White muscle	0.17 ± 0.05	1.3 ± 0.8	0.5 ± 0.3	8.0 ± 4
Fat	0.50 ± 0.09	2.5 ± 1	1.8 ± 0.0	50 ± 1
Heart	14 ± 11	20 ± 9	28 ± 17	204 ± 67
Brain	0.27 ± 0.08	0.89 ± 0.4	1.2 ± 0.5	17 ± 7
Bile	0.04 ± 0.02	0.36 ± 0.3	0.12 ± 0.07	0.69 ± 0.4
Gills	n.d.	n.d.	n.d.	66 ± 40

[a] Mean ± SD of 3–5 catfish. n.d., not determined.

(Rothstein and Hayes, 1960; Webb, 1986). The redistribution of Cd in rats was thought to occur through liberation of hepatic MT because administration of Cd–MT preferentially accumulates in the kidney (Webb, 1986; Sudo *et al.*, 1994). The mechanism for the redistribution of Hg to the liver as well as why a similar redistribution of Cd was not observed in catfish is unclear. Presumably, this could have involved induction of MT in the liver, which scavenged Hg and Cd from ligands of lower affinity for these metals in other tissues. However, it is questionable whether the low dose of Hg and Cd used in our study was sufficient to induce MT.

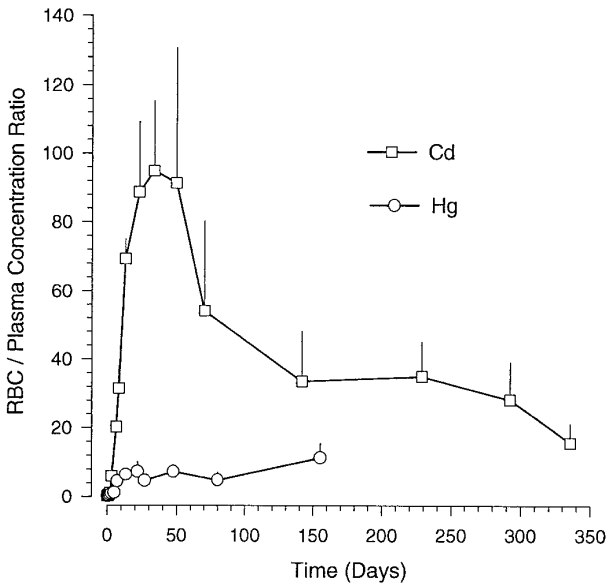

FIG. 8. The red blood cell to plasma concentration ratio of Hg and Cd in channel catfish after intra-arterial injection.

The large V_{ss} for Hg and Cd suggested that these metals had the potential for high accumulation in fishes. However, the bioaccumulation of inorganic Hg and Cd has been reported to be less than 200 after water or oral exposure in fishes (Pentreath, 1976a; Cuvin and Furness, 1988; Harrison and Klaverkamp, 1989). The estimates of biological half-life obtained in our study were longer than those previously reported for these metals in fishes (Weisbart, 1973; De Freitas *et al.*, 1974; Pentreath, 1976a, 1977; Harrison and Klaverkamp, 1989). The longer biological half-life estimates reported in the present study appear to reflect the advantages of simultaneous measurement of blood and whole animal activity of metals in estimating the biological half-life. Also, we characterized the elimination and distribution of Cd using a lower dose that was more representative of tissue concentrations found in natural populations of catfish (McCoy *et al.*, 1995). That accumulation of inorganic Hg and Cd by fishes in the environment is not higher appears to be due to the low bioavailability of these metals from food and water, as our results indicated that fishes have a high capacity for storage and a limited ability to excrete these metals. Thus, a significant factor in determining the accumulation or total body burden of these metals in fishes would be the duration of

exposure because the time required to reach an equilibrium concentration with contaminated food and water may be a significant portion of the life span of the animal.

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REFERENCES

Bai, S. C., and Gatlin, D. M. (1994). Effects of L-lysine supplementation of diets with different protein levels and sources on channel catfish, *Ictalurus punctatus* (Rafinesque). *Aquacult. Fish. Man.* **25,** 465–474.

Bloom, N. (1993). On the chemical form of mercury in edible fish and marine invertebrate tissue. *Can J. Fish. Aquat. Sci.* **49,** 1010–1017.

Borg, K., Gottofrey, J., and Tjalve, T. (1988). Effects of some chelating agents on the uptake and tissue distribution of $^{203}Hg^{2+}$ in the brown trout (*Salmo trutta*): Studies on ethyl- and isopropylxanthate, diethyl- and diisopropyldithiophosphate, dimethyl- and diethyldithiocarbamate and pyrinethione. *Arch. Toxicol.* **62,** 387–391.

Boxenbaum, H. G., Riegelman, S., and Elashoff, R. M. (1974). Statistical estimations in pharmacokinetics. *J. Pharm. Biopharm.* **2,** 123–149.

Chiou, W. L. (1972). A simple equation to estimate the fraction of drug remaining in the body after an intravenous injection. *J. Pharm. Pharmacol.* **34,** 342–344.

Clarkson, T. W. (1972). The pharmacology of mercury compounds. *Annu. Rev. Pharmacol.* **12,** 375–406.

Cuvin, M. L. A., and Furness, R. W. (1988). Uptake and elimination of inorganic mercury and selenium by minnows *Phoxinus phoxinus*. *Aquat. Toxicol.* **13,** 205–216.

De Freitas, A. S. W., Qadri, S. V., and Case, B. E. (1974). Origins and fate of mercury compounds in fish. In *Proceedings of the International Conference on Transport of Persistent Chemicals in Aquatic Ecosystems,* Section III, pp. 31–36. Ottawa, Canada.

Fitzgerald, W. F., and Clarkson, T. W. (1991). Mercury and monomethylmercury: Present and future concerns. *Environ. Health Perspect.* **96,** 159–166.

Friberg, L., Elinder, C. G., and Kjellstrom, T. (1992). *Cadmium.* WHO, Geneva.

Garty, M., Wong, K-L., and Klaassen, C. D. (1981). Redistribution of cadmium to blood of rats. *Toxicol. Appl. Pharmacol.* **59,** 548–554.

Gibaldi, M., and Perrier, D. (1982). *Pharmacokinetics,* pp. 494. Dekker, New York.

Giblin, F. J., and Massaro, E. J. (1973). Pharmacodynamics of methyl mercury in the rainbow trout (*Salmo gairdneri*): Tissue uptake, distribution and excretion. *Toxicol. Appl. Pharmacol.* **24,** 81–91.

Glynn, A. W. (1991). Cadmium and zinc kinetics in fish: Studies on waterborne ^{109}Cd and ^{65}Zn turnover and intracellular distribution in minnows, *Phoxinus phoxinus. Pharmacol. Toxicol.* **69,** 485–491.

Gregus, Z., and Klaassen, C. D. (1986). Disposition of metals in rats: A comparative study of fecal, urinary, and biliary excretion and tissue distribution of eighteen metals. *Toxicol. Appl. Pharmacol.* **85,** 24–38.

Harrison, S. E., and Klaverkamp, J. F. (1989). Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Corregonus clupeaformis* Mitchill). *Environ. Toxicol. Chem.* **8,** 87–97.

Holmes, W. N., and Donaldson, E. M. (1969). The body compartments and the distribution of electrolytes. In *Fish Physiology* (W. S. Hoar and D. J. Randall, Eds.), Vol. 1, pp. 1–89.

Houston, A. H. (1990). Blood and circulation. In *Methods for Fish Biology* (C. B. Schreck and P. B. Moyle, Eds.), pp. 273–322. American Fisheries Society, Bethesda, MD.

Houston, A. H., DeWilde, M. A., and Madden, J. A. (1969). Some physiological consequences of aortic catherization in the brook trout, *Salvelinus fontinalis. J. Fish Res. Bd. Can.* **26,** 1847–1856.

Huckabee, J. W., Elwood, J. W., and Hildebrand, S. G. (1979). Accumulation of mercury in freshwater biota. In *The Biogeochemistry of Mercury in the Environment* (J. O. Nriagu Ed.), pp. 277–302. Elsevier/North-Holland, New York.

Kitzman, J. V., Holley, J. H., and Huber, W. G. (1988). Sample collection techniques in the channel catfish during prolonged pharmacokinetic experiments. *Vet. Human Toxicol.* **30,** 12–13.

Mance, G. (1987). *Pollution Threat of Heavy Metals in Aquatic Environments.* Elsevier, New York.

McCoy, C. P., O'Hara, T. M., Bennett, L. W., and Boyle, C. R. (1995). Liver and kidney concentrations of zinc, copper and cadmium in channel catfish (*Ictalurus punctatus*): Variations due to size, season and health status. *Vet. Human Toxicol.* **37,** 11–15.

McKim, J. M., Nichols, J. W., Lien, G. J., and Bertelsen, S. L. (1994). Respiratory–cardiovascular physiology and chloroethane gill flux in the channel catfish *Ictalurus punctatus. J. Fish Biol.* **44,** 527–547.

Metzler, C. M., Elfring, G. L., and McEwen, A. J. (1974). A package of computer programs for pharmacokinetic modeling. *Biometrics* **30,** 562–563.

Nielsen, J. B. (1992). Toxicokinetics of mercuric chloride and methylmercuric chloride in mice. *J. Toxicol. Environ. Health* **37,** 85–122.

Nordberg, G. F., Piscator, M., and Nordberg, M. (1971). On the distribution of cadmium in blood. *Acta Pharmacol. Toxicol.* **30,** 289–295.

Norrgren-Karlsson, L., and Runn, P. (1985). Cadmium dynamics in fish: Pulse studies with ^{109}Cd in female zebrafish, *Brachydanio rerio. J. Fish Biol.* **27,** 571–581.

Nriagu, J. O., and Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* **12,** 134–139.

Olson, K. R., Bergman, H. L., and Fromm, P. O. (1973). Uptake of methyl mercuric chloride and mercuric chloride by trout: A study of uptake pathways into the whole animal and uptake by erythrocytes in vitro. *J. Fish. Res. Bd. Can.* **30,** 1293–1299.

Pentreath, R. J. (1976a). The accumulation of mercury from food by the plaice, *Pleuronectes platessa* L. *J. Exp. Mar. Biol. Ecol.* **25,** 51–65.

Pentreath, R. J. (1976b). The accumulation of inorganic mercury from seawater by the plaice, *Pleuronectes platessa* L. *J. Exp. Mar. Biol. Ecol.* **24,** 103–119.

Pentreath, R. J. (1977). The accumulation of cadmium by the plaice, *Pleuronectes platessa* L. and the thornback ray, *Raja clavata* L. *J. Exp. Mar. Biol. Ecol.* **30,** 223–232.

Roesijadi, G. (1992). Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquat. Toxicol.* **22,** 81–114.

Rothstein, A., and Hayes, A. D. (1960). The metabolism of mercury in the rat studied by isotope techniques. *J. Pharmacol. Exp. Ther.* **130,** 166–176.

Sorenson, E. M. B. (1991). *Metal Poisoning in Fish.* CRC Press, Boca Raton, FL.

Stary, J., Havlik, B., Kratzer, K., Prasilova, J., and Hanusova, J. (1981).

Mercury circulation in aquatic environment. Part 4: The accumulation of inorganic mercury and phenylmercury by Fish (*Poecilia reticulata* (Peters)). *Acta Hydrochim. Hydrobiol.* **9,** 545–553.

Stehly, G. R., and Plakas, S. M. (1993). Pharmacokinetics, tissue distribution and metabolism of nitrofurantoin in channel catfish. *Aquaculture* **113,** 1–10.

Sudo, J-I., Hayashi, T., Terui, J., Soyama, M., Fukata, M., and Kakuno, K. (1994). Kinetics of Cd^{2+} in plasma, liver and kidneys after single intravenous injection of Cd-metallothionein-II. *Eur. J. Pharmacol.* **270,** 229–235.

Tanaka, K., Min, K-S., Onosaka, S., Fukuhara, C., and Ueda, M. (1985). The origin of metallothionein in red blood cells. *Toxicol. Appl. Pharmacol.* **78,** 63–68.

Thompson, N. T., and Klaassen, C. D. (1983). Disposition of metals after portal and systemic administration to rats. *Toxicol. Appl. Pharmacol.* **68,** 442–450.

Tucker, C. S., and Robinson, E. H. (1990). *Channel Catfish Farming Handbook.* Van Nostrand–Reinhold, New York.

U.S. Environmental Protection Agency (USEPA) (1978). U.S. Environmental Protection Agency: Environmental Monitoring Series. Quality assurance guidelines for biological testing. Environmental Monitoring and Support Laboratory, Cincinnati, OH, EPA-600/4-78-043.

Weatherly, A. H., and Gill, H. S. (1987). *The Biology of Fish Growth.* Academic Press, Orlando, FL.

Webb, M. (1986). Role of metallothionein in cadmium metabolism. In *Cadmium* (E. C. Foulkes, Ed.). Springer-Verlag, New York.

Weisbart, M. (1973). The distribution and tissue retention of mercury-203 in the goldfish (*Carassius auratus*). *Can. J. Zool.* **51,** 143–150.

Williams, D. R., and Giesy, J. P. (1978). Relative importance of food and winter sources to cadmium uptake by *Gambusia affinis* (Poeciliidae). *Environ. Res.* **16,** 326–332.