Pharmacokinetics of Intravascularly Administered [65]Zinc in Channel Catfish (*Ictalurus punctatus*)

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Comparison was made of the pharmacokinetics of the radioisotope [65]Zinc ([65]Zn) in blood, plasma, and whole body of adult channel catfish (*Ictalurus punctatus*) following intravascular (iv) administration. A two-compartment model described the pharmacokinetics of [65]Zn in plasma and blood during the first 40 days following iv administration, but was unable to describe the long-term disposition of [65]Zn. Whole-body counting revealed that approximately half of the [65]Zn dose was sequestered in a slowly exchangeable pool with a half-life of 1.5 years. Greater than 99% of the circulating [65]Zn was bound to plasma proteins, whereas there was less than 1% binding to red blood cells. Synthesis of the results for channel catfish and existing data in other species indicates three phases in the pharmacokinetics of zinc. The first phase consists of initial distribution outside the vascular system to kidney, liver, and other organs (alpha phase in blood and plasma; $t_{1/2}$ of 4 to 5 h). The second phase involves distribution from organs to a slowly exchangeable zinc pool, likely consisting of bone (beta phase in blood and plasma; alpha phase in whole body; $t_{1/2}$ of 4 to 20 days). The third phase appears to involve a slow turnover of sequestered zinc ($t_{1/2}$ greater than 1 year). Blood sampling or short-term whole-body measurements will underestimate the persistence of zinc in fish, thus prolonged sampling and measurement of whole-body concentrations are necessary to characterize the pharmacokinetics of zinc.   © 2000 Academic Press

Key Words: catfish; compartmental model; half-life; zinc.

INTRODUCTION

Zinc is an essential nutrient in fish and a toxic contaminant of aquatic environments impacted by mining or metal processing operations (Eisler, 1993). In mammalian species, zinc initially accumulates in the liver, kidney, pancreas, and spleen within the first 2 to 24 h of exposure and then is redistributed to bone (Gregus and Klaasen, 1986; Hammond and Beliles, 1980; Rubini *et al.*, 1961; Guillard *et al.*, 1984). Intravascularly (iv) administered zinc in rats was primarily excreted in the feces (31% of dose) during the first 4 days of elimination, and renal and biliary excretion were minor (≤2% of dose) (Gregus and Klaassen, 1986).

Previous studies have reported either relatively short (a few weeks) or long (several months) half-lives of zinc in both mammals and fish, depending on the test species and experimental design (Rubini *et al.*, 1961; Shulman *et al.*, 1961; Andermann and Dietz, 1982; Alsop *et al.*, 1999). For example, whole-body measurements of the half-life of [65]Zn in small fish range from 13 days in silversides *Menidia menidia* (Shulman *et al.*, 1961) to greater than 6 months in mosquitofish *Gambusia holbrooki* (*formerly*, *G. affinis*) (Willis and Jones, 1977; Newman and Mitz, 1988).

The objective of this study was to investigate the pharmacokinetics of iv-administered [65]Zn in the blood, plasma, and whole body of adult channel catfish. The pharmacokinetics of iv-administrated zinc has not been previously determined in fish, but iv dosing has been used in previous pharmacokinetic studies of other chemicals in channel catfish (e.g., Barron *et al.*, 1991; Schultz *et al.*, 1996; Schultz and Newman, 1997). Intravascular administration allows pharmacokinetic determinations in the absence of any confounding effects of absorption dynamics and tissue binding at the site of absorption. A nontoxic dose of the radioisotope [65]Zn was administered because of the high concentrations of endogenous zinc in fish (e.g., 3 to > 10 mg/kg; NAS, 1979). For example, fish contain 3 to greater than 100 mg Zn/kg (Eisler, 1993), which may mask the kinetics of low-dose zinc administration (Alsop *et al.*, 1999). Fish were sampled for nearly 1 year to characterize the persistence of [65]Zn in the channel catfish. Also investigated was the binding of [65]Zn to washed red blood cells (RBCs) and to plasma proteins (in the absence of RBCs) because other metals exhibit high binding to these blood components (e.g., Schultz *et al.*, 1996).

MATERIALS AND METHODS

Fish and Water Quality

Adult channel catfish (0.13 to 0.16 kg) of mixed sex were obtained from Orangeburg Aquaculture (Cordova, SC) and

maintained in 400-liter recirculating water, fiberglass aquarium (LS 700, Frigid Units, Toledo, OH) containing reconstituted hard water (EPA, 1978) and 1‰ (w/v) NaCl. The loading density of catfish in the aquaria was below 5 g/liter Half of the aquarium water was replaced biweekly with aerated, reconstituted water. Chemical characteristics of the freshly prepared water were as follows: 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 levels in the aquaria were regularly monitored to ensure that the concentration did not exceed 0.5 mg/liter. 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). Immediately upon arrival, the catfish received a 2-h treatment in a 0.25 mg/liter malachite green (Sigma Chemical) solution and were held a minimum of 30 days before use.

Surgical Procedure

Each catfish was fitted with a dorsal aortic cannula using methods described in Schultz et al. (1996). 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 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 h before dosing.

Dosing and Sampling

The $^{65}ZnCl_2$ was obtained from Amersham, Inc. (Arlington Heights, IL). Catfish were injected intraaortically with $^{65}ZnCl_2$ (12 µg/kg) dissolved in a modified Cortland saline (Schulz and Newman, 1996). Two protocols were used sequentially to obtain blood samples and whole-animal activity estimates. Initially, serial blood samples were removed via the cannula at 0.3, 0.7, 1, 1.5, 2, 3, 5, 8, 14, 25, 50, 80, and 175 h after injection. The catfish were not fed during the first 2 weeks of sampling. After the blood sample had been removed at 50 h, the whole-animal activity of ^{65}Zn was quantified by placing the unanesthetized catfish for 3 min in a 4-liter Marinelli beaker (GA-MA and Associates, Inc.) containing 1 liter of water. The catfish was returned to its cage in the aquarium immediately after counting. At 175 h, the catfish was 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 was then placed in the Marinelli beaker for counting.

After 175 h, the cannula was removed while the catfish were anesthetized, and later blood samples were obtained from the dorsal aorta of the anesthetized catfish using a 25-gauge needle and a 1-ml syringe immediately before whole-animal counting. Such sampling continued for 323 days. Periodically, anesthetized catfish were weighed before placement in the Marinelli beaker. After 175 h, 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 h after the last feeding period.

Aliquots of whole blood, plasma, and RBCs were separated from each blood sample, as described in Schultz et al. (1996). After removal of a blood sample, a fraction was used for whole-blood determination and the remainder centrifuged for 5 min at 2000g 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; the supernatant was discarded after each wash. The total quantity of blood removed during the initial 196 h was less than 10% of the estimated blood volume (assumed to be 4% of body weight). After 196 h, no more than 3% of the estimated blood volume was removed for each sample.

Gamma Counting and Determination of Zn

The ^{65}Zn activities of blood products (plasma, blood, RBCs) were determined by counting the samples to a 2σ error of 1% in an automated 7.6-cm-wide × 8.3-cm-high well-type NaI(Tl) gamma counter (Packard Auto-gamma Model 5530, Packard Instrument Co.). Counts were corrected for radioactive decay to a standard reference date supplied by Amersham, Inc., and converted to values of stable zinc using specific activities calculated from the ^{65}Zn standards. The ^{65}Zn activities of the whole fish were estimated using a 7.6-cm-wide × 7.6-cm-high well-type NaI(Tl) solid scintillator detector/photomultiplier and multichannel analyzer (Canberra Series 85, Canberra, Meridan, CT). The Marinelli beaker containing the catfish was placed on the detector and the gamma emissions were counted for 3 min. Background count rates were determined using a water-filled Marinelli beaker with the same geometry as used with catfish. The net count rates were corrected for radioactive decay to the same reference date used previously for the blood products. At completion of the study, the catfish were killed by anesthetic overdose and placed in the Marinelli beaker, and the whole-body count rate was estimated from three separate measurements of 5 min each. Immediately afterward, the catfish were homogenized in a Waring blender and aliquots of the homogenate counted in the Packard NaI(Tl) 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 which 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 of hct values from cannulated catfish (Mean $\pm$ SD: 17.0 $\pm$ 5%; $n = 10$) and is similar to values reported by other researchers (e.g., McKim *et al.*, 1994). The hct of blood samples obtained under anesthesia was measured using heparinized microhematocrit tubes, and Zn concentrations in whole blood and washed RBCs were adjusted to a hct value of 17% by the equation

$$C_b = C_o - ((\text{hct} - 0.17)^*C_{RBC}) + ((\text{hct} - 0.17)^*C_p), \quad (1)$$

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

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

where X_{RBC} = amount of Zn in the washed RBCs (ng), and blood volume was the fraction of the blood sample used to obtain the washed RBCs.

Plasma Binding

Binding of ^{65}Zn to plasma proteins was determined by centrifugation (1500g) of plasma samples through Centrifree (Amicon, Inc., Beverly, MA) filtration tubes (30,000-Da cutoff). The percentage bound was calculated from the ratio of radioactivity in the plasma filtrate and the radioactivity in the original plasma sample:

$$\% \text{ bound} = [1 - (\text{dpm in filtrate/dpm in sample})] \times 100. \quad (3)$$

Pharmacokinetics

The pharmacokinetics of ^{65}Zn were evaluated by modeling mean concentrations in blood ($n = 1$ to 6), plasma ($n = 2$ to 6), and whole body ($n = 4$) using WinNonlin software (Statistical Consultants, Inc., Apex, NC). Multicompartmental models with clearance constants as parameters were used in fitting the data (Barron *et al.*, 1990). The most appropriate compartmental model for each data set

was determined from the uncertainty of the estimated parameters [i.e., coefficient of variation for total body clearance (Cl_b), volume of distribution of the central compartment (V_1), and the sum of all compartment volumes (V_{ss}), mean residence time (MRT), and terminal elimination half-life ($t_{1/2}$)], and the absence of systematic bias in plots of observed and model predicted zinc concentrations.

RESULTS

Blood and Plasma Pharmacokinetics

Mean blood concentrations of ^{65}Zn exhibited a biexponential decline from 318 to 11 ng/ml during the first 37 days following iv administration (Fig. 1). Mean plasma concentrations of ^{65}Zn also exhibited a biexponential decline, decreasing from 340 (0.01 days) to 8 (37 days) ng/ml (Fig. 2). Mean concentrations of ^{65}Zn in red blood cells were very low and relatively constant during the first 37 days, ranging from 4.5 to 15 ng/ml (Fig. 2). Concentrations of ^{65}Zn in a single fish sampled for 323 days declined to nearly nondetectable levels (blood: 2.5 ng/ml; plasma: 2.1 ng/ml; RBCs: 0.5 ng/ml) (data not provided). These samples were counted to a 2-sigma level of confidence (98%) to ensure the accuracy of the low-level measurements. The ^{65}Zn in blood was greater than 99% bound to plasma proteins (data not shown). A two-compartment model best described the ^{65}Zn pharmacokinetics in the blood and plasma of channel catfish during the first 37 days following iv administration. The elimination from the vascular system (Cl_b), extent of tissue distribution (V_1, V_{ss}), and persistence in the vascular system (MRT, $t_{1/2}$) of ^{65}Zn was similar for both plasma and blood determinations (Table 1). The half-life of the first distributive (alpha) phase of ^{65}Zn in blood and plasma was approximately 4.5 h. Preliminary modeling and sample data for
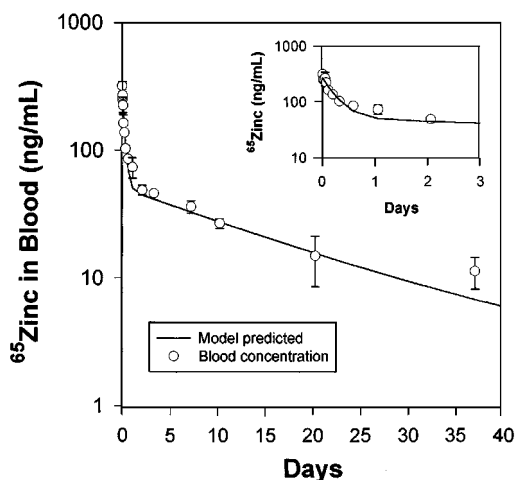

FIG. 1. Concentrations of ^{65}Zn in blood of channel catfish. Symbols indicate the observed data and lines indicate the pharmacokinetic model-predicted values. Inset: blood concentrations of ^{65}Zn for the first 3 days following iv administration.
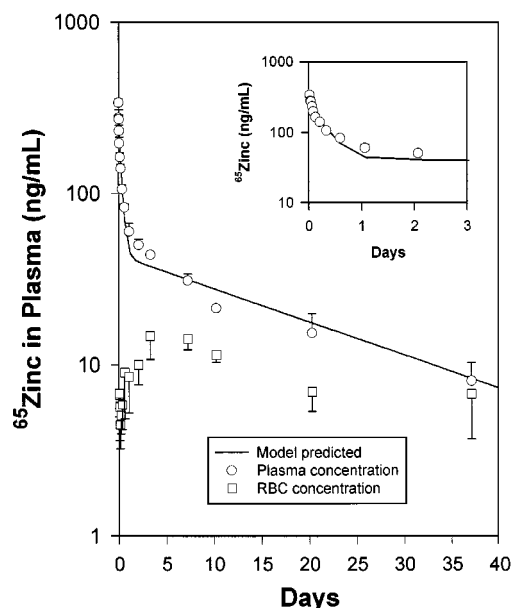
FIG. 2. Concentrations of ^{65}Zn in plasma (circles) and red blood cells (squares) in channel catfish. Line indicates the pharmacokinetic model-predicted concentration of ^{65}Zn in plasma. Inset: plasma concentrations for the first 3 days following iv adminstration.

a single catfish indicated that elimination from the vascular system was minimal after 50 h and that blood and plasma concentrations declined to a nearly constant value of 2 to 3 ng/ml.

Whole-Body Pharmacokinetics

Mean concentrations of ^{65}Zn in whole-body samples of channel catfish exhibited a biexponential decline from 12.3 to 6.6 µg/kg during a 323 day elimination period following iv administration (Fig. 3). Catfish maintained >90% of their surgery weight during the nearly year-long study. A two-compartment model also best described the pharmacokinetics of ^{65}Zn in the whole-body of catfish. However,
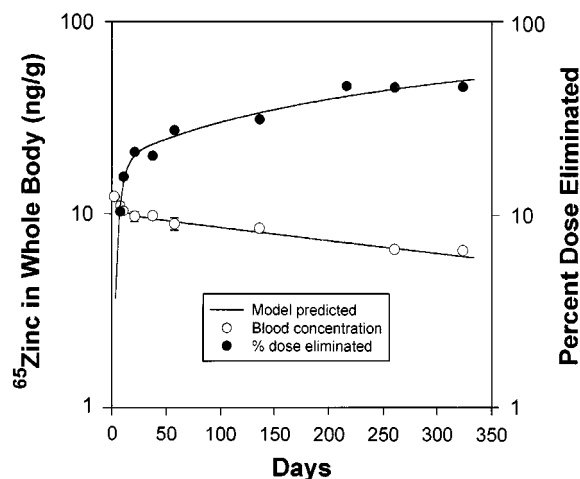

FIG. 3. Concentrations of ^{65}Zn in whole body of channel catfish (open circles: observed values; line: model predicted values), and the percentage of the dose eliminated (solid circles: observed data; line: model predicted values).

in contrast to blood and plasma, whole-body concentrations of ^{65}Zn exhibited a more prolonged distribution period (alpha phase $t_{1/2}$ of 4.3 days) and extremely slow elimination ($t_{1/2}$ of 1.5 years). Whole-body pharmacokinetics also exhibited a relatively larger volume of distribution than ^{65}Zn in blood and plasma (Table 1). Figure 3 shows that the percentage of the iv dose eliminated from channel catfish was 20% after 37 days, and 48% after 323 days.

DISCUSSION

A two-compartment model described the pharmacokinetics of ^{65}Zn in plasma and blood during the first 37 days following iv administration, but was unable to describe the long-term disposition of ^{65}Zn in channel catfish. Preliminary modeling and data for a single fish indicated that elimination from the vascular system was minimal after 50 h and that blood concentrations declined to a nearly constant value of 2 to 3 ng/ml. Whole-body counting revealed that approximately half of the ^{65}Zn dose was sequestered in a slowly exchangeable pool with a half-life of 1.5 years. Blood and plasma levels of ^{65}Zn exhibited a 95% decline in the first 20 days due to distribution outside of the vascular system, rather than elimination. The majority of ^{65}Zn loss from the vascular system occurred within the first 24 h. For example, plasma concentrations declined 80% within 24 h of iv administration. In contrast, approximately 100% of the dose was retained in the whole body of channel catfish for the first 48 h and greater than 80% of the dose was still in the fish 20 days following iv administration.

Previous studies have reported either relatively short (a few weeks) or long (several months) half-lives of zinc in both mammals and fish, depending on the test species and

TABLE 1
Pharmacokinetic Parameters in Channel Catfish (SE)

Parameter	Units	Tissue		
		Plasma	Blood	Whole body
Cl_b	ml/d/kg	12 (0.7)	9.6 (1.0)	—
	g/d/kg	—	—	46 (4.8)
V_1	ml/kg	44 (4.1)	44 (5.1)	—
	g/kg	—	—	920 (60)
V_{ss}	ml/kg	250 (21)	250 (28)	—
	g/kg	—	—	1,200 (40)
MRT	days	21 (1.8)	26 (3.7)	650 (87)
alpha $t_{1/2}$	days	0.2 (0.04)	0.2 (0.05)	4.3 (2.1)
beta $t_{1/2}$	days	16 (1.3)	19 (2.7)	452 (60)

Species	Tissue	Dose route	Half-life (days)	Source
Channel catfish	Whole body	iv	450	This study
	Plasma	iv	16	This study
Mosquito fish (*Gambusia holbrooki*)	Whole body	Environment	235	Willis and Jones (1977)
	Whole body	Water	215	Newman and Mitz (1988)
Mummichog (*Fundulus heeroclitus*)	Whole body	Oral	58	Shulman *et al.* (1961)
Menidia menidia	Whole body	Oral	13	Shulman *et al.* (1961)
Tautogolabrus adsoersus	Whole body	Oral	45	Shulman *et al.* (1961)
Rainbow trout (*Oncorhynchus mykiss*)	Whole body	Oral	140	Nakatani (1966)
	Whole body	Water	150	Alsop *et al.* (1999)
Micropogon undulatus	Not specified	ip	6.5	NAS (1979)
Flatfish (*Pleuronectes platessa*)	Not specified	Water	295–313	NAS (1979)
Mouse	Whole body	ip	10	Cotzias *et al.* (1962)
	Plasma	ip	7	Rubini *et al.* (1961)
Dog	Plasma	iv	> 60	Rubini *et al.* (1961)
Rabbit	Plasma	iv	0.2–0.5	Andermann and Dietz (1982)
	Serum	iv	0.1	Guillard *et al.* (1984)

experimental design (Rubini *et al.*, 1961; Shulman *et al.*, 1961; Andermann and Dietz, 1982; Alsop *et al.*, 1999) (Table 2). For example, whole-body measurements of the half-life of ^{65}Zn in fish range from 13 days to greater than 6 months (Shulman *et al.*, 1961; Willis and Jones, 1977; Newman and Mitz, 1988). In agreement with the current results, Nakatani (1966) observed a rapid loss of zinc in blood, and very slow loss of whole-body levels (e.g., $t_{1/2}$ of 5 months). Alsop *et al.* (1999) reported fast and slow turnover pools of zinc in rainbow trout blood, but only a slow turnover pool was evident in the whole body (turnover rate of 216 μg/kg per day). Mosquitofish at steady state with zinc^{65} exhibited three reservoirs of zinc containing 9, 4, and 91% of zinc, and the terminal elimination half-life was 235 days (Willis and Jones, 1977). The ^{65}Zn in the blood of mice and rats was less than 0.01% of whole-body radioactivity, indicating rapid extravascular distribution (Rubini *et al.*, 1961). Total-body turnover of ^{65}Zn in mice was initially rapid (approximate half-life of 6 days) followed by a slower decline (> 30 day half-life) (Cotzias *et al.*, 1962). Elimination of whole-body ^{65}Zn from mice was accelerated by administration of a metabolic load of stable zinc, whereas administration of metabolic loads of cadmium, copper, or gallium had no effect (Cotzias *et al.*, 1962).

The model-estimated volume of the central compartment (V_1) determined in channel catfish blood (44 ml/kg) was only slightly larger than the vascular volume in fish (Olson, 1992). The steady-state volume of distribution (V_{ss}) determined from intravascular kinetics was 25% of body weight, and thus may comprise tissues that are well perfused with blood such as the visceral organs and red muscle (Schultz *et al.*, 1999). Bone may represent the slow turnover pool in both fish and mammals. In rats, injected ^{65}Zn was first deposited in the pancreas, liver, and spleen, then a large proportion was transferred to bone (Rubini *et al.*, 1961). Six months after zinc administration in rainbow trout (*Oncorhynchus mykiss*), bone had the highest zinc concentration of any tissue and the slowest elimination (Nakatani, 1966). Rapidly growing juvenile chinook salmon (*Oncorhynchus tshawytscha*) accumulated ^{65}Zn from water and retained nearly all of it for 63 days after transfer to uncontaminated media; most of the zinc was translocated to the vertebral column, head, and visceral mass (Joyner and Eisler, 1961). Juvenile channel catfish fed 200 mg zinc for 12 weeks had elevated bone zinc levels (359 mg/kg dw) relative to control fish (254 mg/kg dw) (Gatlin *et al.*, 1989). Bently (1991) also reported high concentrations of zinc in bone of channel catfish (59 mg/kg ww), suggesting that 30 to 40% of zinc residues were in bone (calculated using a relative bone weight of 18% of body weight in channel catfish; Schultz *et al.*, 1999). The outer surface of the bone seems to be an ion-exchange medium capable of sequestering large quantities of metal ions through the process of overlayering by growing bone.

The pharmacokinetics of ^{65}Zn in blood were nearly identical to plasma because of minimal binding to RBCs. Greater than 99% of the circulating ^{65}Zn was bound to plasma proteins, whereas there was less than 1% binding to red blood cells. Bently (1991) previously reported high-affinity binding of zinc to specific plasma proteins in channel catfish. Concentrations of ^{65}Zn were near detection limits (0.5 ng/ml) but appeared to represent a low, relatively constant, level of binding to RBCs (i.e., 1 to 15 ng/ml). In contrast, several other metals in channel cat-fish exhibit substantially higher binding to RBCs (Schultz *et al.*, 1996, Schultz and Newman, 1997). Current results with zinc are in

contrast to studies in rodents that have suggested that zinc concentrates in the membrane of the RBC (Bettger *et al.*, 1987).

CONCLUSION

The results of this study confirm the long biological half-life of zinc in fish, and suggest that previous reports of a limited persistence of metals in fish should be interpreted relative to the experimental design and measurement methods used. Synthesis of the results for channel catfish and existing data in other species indicates three phases in the pharmacokinetics of zinc. The first phase consists of initial distribution outside the vascular system to organs such as kidney, liver, and spleen (alpha phase in blood and plasma; $t_{1/2}$ of 4 to 5 h). The second phase involves distribution from organs to a slowly exchangeable zinc pool, likely consisting of bone (beta phase in blood and plasma; alpha phase in whole body; $t_{1/2}$ of 4 to 20 days). The third phase appears to involve a slow turnover of sequestered zinc ($t_{1/2}$ greater than 1 year). In agreement, three phases of zinc disposition are evident from whole-body measurements in mosquitofish (Willis and Jones, 1977) and plasma measurements in dogs (Rubini *et al.*, 1961). Blood sampling or short-term whole-body measurements will underestimate the persistence of zinc in fish, thus prolonged sampling and measurement of whole body concentrations are necessary to characterize the pharmacokinetics of zinc.

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