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Biokinetics of Coping Mechanism of Freshwater tilapia following Exposure to Waterborne and Dietary Copper

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***Abstract*—**The purpose of this study was to understand the main sources of copper (Cu) accumulation in target organs of tilapia (*Oreochromis mossambicus*) and to investigate how the organism mediate the process of Cu accumulation under prolonged conditions. By measuring both dietary and waterborne Cu accumulation and total concentrations in tilapia with biokinetic modeling approach, we were able to clarify the biokinetic coping mechanisms for the long term Cu accumulation. This study showed that water and food are both the major source of Cu for the muscle and liver of tilapia. This implied that control the Cu concentration in these two routes will be correlated to the Cu bioavailability for tilapia. We found that exposure duration and level of waterborne Cu drove the Cu accumulation in tilapia. The ability for Cu biouptake and depuration in organs of tilapia were actively mediated under prolonged exposure conditions. Generally, the uptake rate, depuration rate and net bioaccumulation ability in all selected organs decreased with the increasing level of waterborne Cu and extension of exposure duration. Muscle tissues accounted for over 50% of the total accumulated Cu and played a key role in buffering the Cu burden in the initial period of exposure, alternatively, the liver acted a more important role in the storage of Cu with the extension of exposures. We concluded that assumption of the constant biokinetic rates could lead to incorrect predictions with overestimating the long-term Cu accumulation in ecotoxicological risk assessments.

***Keywords*—**Biokinetics, Chronic exposure, Copper, Coping mechanism, Tilapia

I. Introduction

ONE of the major challenges for assessing the potential heavy metal stress to aquatic organisms is to predict explicitly the internal active dose in specific organs. Aquatic organisms subject to temporal fluctuations of bulk contaminants caused by sewage elimination, surface water runoff and meteorological-associated hydrologic dilution, dispersion or condensation, as well as biogeochemical processes which can variegate of time-variant exposure scenarios ([1]-[3]). Physiological acclimation and mediations, including changes in metal uptake rate, rate constant of loss and the recovery rate of damaged target organ or tissue, might be developed to overcome potential toxic tresses from contaminants, especially during prolonged exposure to sublethal or chronic concentrations ([4]-[7]). McGeer et al. ([8]) showed that an inverse relationship exists between bioconcentration factor (BCF) of aquatic biota and waterborne metal concentrations. Liao et al. ([9]) indicated that the field tilapia featured with higher arsenic (As) accumulation ability (BCF = 143–421) than those adopted in their 7-d lab bioaccumulation assays (BCF = 1.04–4.19), in which the lab group suffered with nearly 30 times higher waterborne as concentrations than the field group. Wood et al. ([10]) indicated that the internal accumulation of metal in fish during chronic exposures were different than during acute exposures. They found that elevated mercury concentration was observed in intestine during 24-h exposure and were not detectable in 6-d exposures. On the contrary, several studies supported that fish decreased ([4][11]), or unchanged their ability ([12][13]) to accumulate metals after previous metal exposure acclimations. Thus a further understanding in physiological mediation for biokinetic (BK) processes (i.e., uptake, accumulation, and depuration) of chemicals during chronic exposures is critical to assess the metal exposure risk for indigenous species populations ([8][14]).

Organisms might alter their ability to uptake or eliminate the metal by physiological and biochemical modifications (e.g. restore the impaired branchial Na^+ transportation) and resulted in the change in chemical bioavailability ([15][7]). These factors might limit the model for accounting for uncertainties caused by these factors. Accordingly, using the BK parameters estimated from a single concentration-invariant treatment to extrapolate the metal burden in different exposure circumstances might be far away from the real chemical stress encountered by aquatic organisms in their natural environments.

The tilapia *Oreochromis mossambicus* is a traditional food fish for the people of Taiwan. It is also one of the most abundant species in local freshwater and estuary ecosystems in Taiwan. Tilapia are commonly existing in both pristine and metal polluted ecosystems, and they have been the target species in several earlier studies inspecting the metal toxicokinetics, toxicodynamics and physiological responses ([16]-[18]). Copper (Cu) is an essential element in aquatic ecosystems as well as being nondegradable and cumulative pollutant, which exert a wide range of pathologic effects on fish and other aquatic organisms ([19][20]). Cooper sulfate is commonly applied to aquaculture ponds in Taiwan to eradicate filamentous algae as well as blue-green algae ([20]). The total Cu concentration in fish farm water ranged from 0.01 to 0.12 μg mL^{-1}, some of which far exceeding the permitted maximum Cu level for aquacultural water (0.03μg mL^{-1}, [21]). A significant increase in Cu levels in pond water could produce severe effects on the health of farmed tilapia and even cause a health problem to people who consume the farmed tilapia.

Metal toxicity to aquatic organisms have previously been found to relate to organ-specific biokinetic especially in chronic exposure circumstances ([22]-[24]). Yet the current studies rarely consider biokinetic mediation of exposed organisms for chronic toxicity predictions. Despite some studies have assessed the physiological responses upon Cu exposure in juvenile tilapia ([18][20]), however, these studies lacked a

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reliable data existing on actual uptake and elimination processes in specific organs of tilapia, especially in prolonged exposure scenarios. Farmed fish are exposed to heavy metals through both ambient water and food simultaneously, however, the relatively importance of aqueous and dietary sources contributing the metal residue in aquatic organisms is lacked of agreement among researchers because of the difference in experimental designs. For example, some studies used artificial, metal-spiked feeds ([11][10][25]) and other researchers have assessed the importance of dietary metal by using contaminated live prey ([26][24][27]) as the food-borne exposure route. The relative fraction of soluble metal within feed (or prey) might also account for these inconsistencies because soluble metal tends to be more bioavailable to the consumer than these binding with insoluble fractions ([28][29]).

In the present study, we sought to characterize the change in the ability of Cu uptake and elimination of tilapia organs or tissues along a Cu concentration gradient in prolonging waterborne exposures by biokinetic modeling approach. The purposes of this study were (1) to determine the relative contribution of waterborne and dietary Cu for organ-specific Cu burdens in farmed juvenile tilapia, (2) to investigate the relationship between the time course of exposure duration, waterborne Cu concentration and the change in organ-specific ability of Cu uptake, elimination, and accumulation, and (3) to clarify the biokinetic coping mechanisms for mediating the long-term Cu accumulation.

II. MATERIAL AND METHODS

A. Bioaccumulation bioassays

Juvenile male tilapia (*O. mossambicus*) with ages of 4-5 moths (mean body length = 13.9 ± 1.7 cm (mean ± SD) and mean weight = 20.2 ± 7.3 g wet wt.) were hatched in laboratory. Fish were allowed to acclimate in tap water at least 14 d before the beginning of exposure tests. No mortality recorded during the acclimatization and no weight losses were observed. Two treatment regimes were assigned to investigate, firstly, the time series of Cu bioaccumulations in selected organs of *O. mossambicus* from both dietary and waterborne Cu simultaneously, and secondly to determine the Cu accumulation kinetics in fish organs from dietary exposures only. Copper stock solutions were prepared by dissolving a calculated amount of $CuSO_4 \cdot H_2O$ (Shimada Chemical Works, Japan) in double-deionized water. Stock solutions was diluted to the nominal concentrations with local tap water (tap water conditions were Ca^{2+} of 62.02 ± 3.39 μg mL^{-1}, Mg^{2+} of 13.27 ± 0.5 μg mL^{-1}, Na^{+} of 9.47 ± 0.71 μg mL^{-1}, K^{+} of 2.97 ± 0.48 μg mL^{-1}, and Fe^{2+} of 11.29 ± 7.31 μg L^{-1}, mean±SD, n = 6). All experiments were carried out in 63 L indoor rectangular glass tank with circulated, aerated tap water at 26-28°C with a photoperiod of 12 h with an intensity of 1400 ± 100 lux.

To investigate the Cu accumulation kinetics form the dietary source only, fish were reared in uncontaminated tap water for 14 d and fed with Cu-spiked commercial fish food at a rate of 4% (dw/ww) of fish biomass everyday. The Cu concentration of commercial fish diet (Tong-Bau Co., Taiwan) was 13.5 ± 0.51 μg g^{-1}. In second series of treatments, tilapia were exposed to Cu concentrations of 0, 0.1, 0.2, 0.3, and 0.6 μg mL^{-1} for 14 days based upon the experimental arrangement of Wu et al. ([20]). The average background waterborne Cu level in control group was 0.011 ± 0.004 μg mL^{-1} and the measured Cu concentration was 0.1 ± 0.02, 0.19 ± 0.04, 0.30 ± 0.08, and 0.58 ± 0.13 μg mL^{-1} (n = 6). Assigned Cu concentrations were around one to two order higher than these measured in field ponds in order to produce significant Cu accumulation and induced possible homeostasis during the bioassays. Uneaten food and feces were siphoned from the bottom of the aquaria to avoid the leaching of Cu from food to water. 30% of the ambient water was replaced daily to avoid the Cu contamination and regression of water quality in these assays.

The food consumption rate (%) and body weight (g) of fish in different treatments were recorded at daily and weekly basis, respectively. The whole Cu solutions were renewed weekly in each tank. Three fish were sequentially harvested from solutions after 0, 0.5, 1, 3, 7, 10, and 14-d of exposure in these two treatments. The fish were rinsed with deionized water and then were anesthetized in pH-neutralized tricaine methane sulfonate (MS-222) (Sigma Chemical Co., St. Louis, MO) solution. The fish were weighted and than sacrificed by a spinal dislocation and then dissected. The gills, alimentary canal, muscle, and liver were collected for metal analyses.

Fish samples were freeze-dried overnight, and then grounded to fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500 mg portion of the powder was digested in 10 mL nitric acid (65% HNO_3, Merck, Geermany) overnight at room temperature. The digested solution was redissolved in 0.2% HNO_3 for quantification of Cu content.

A Perkin-Elmer Model AA-200 atomic absorption spectrophotometer (Perkins-Elmer, Shelton, CT, USA) was used to quantify the total Cu concentration in samples. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference material (Dog fish muscle, DORM-2, NRC-CNRC, Canada). Cu concentrations in each test solution during the experiments were measured 3 times weekly in one selected replicated aquarium. The 10 mL water samples were acidified (pH < 1) with 5 mL 1 N HNO_3 and then stored at -4°C in the dark until they were analyzed. Recovery rate was 94.6±3.6% and the levels of detection were 20 μg Cu L^{-1} for water samples and 20 μg Cu g^{-1} for tissue samples.

B. Data analysis

1. Estimation for organ-specific biokinetic parameters

Time-course of Cu bioaccumulation in aquatic organisms was considered as the net consequence of metal uptake, metal elimination process, and organism growth. Because fish in field are exposed to metals through both water and their diet, thus a predicted model for metal uptake and depuration (i.e., biokinetics) in organism could be described by the following equation:

$$\frac{dC(t)}{dt} = k_{uw}C_w + k_{uf}C_f - \left[\left(k_{ew} + k_{ef} + k_g\right)C(t)\right], \quad (1)$$

Where $C(t)$ is the time-dependent Cu concentration in the selected organ (μg g^{-1}), k_{uw} and k_{uf} are organ-specific uptake rate

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constant for Cu uptake from water (mL g^{-1} d^{-1}) and food (μg g^{-1} d^{-1}), respectively, k_{ew} and k_{ef} are the elimination rate constants (d^{-1}) of metal from water and diet sources, respectively, k_g is the growth rate constant (d^{-1}) and can be calculated from the equation of $k_g = \left[\ln\left(\frac{W_t(t)}{W_0}\right)/dt\right]\times 100$ where $W(t)$ is the biomass of fish in time t, W_0 is the body weight of fish at the beginning of the experiment, t is the time in d, C_w is the waterborne Cu concentration (μg mL^{-1}), and C_f is the Cu concentration in the food of tilapia (μg g^{-1}). The method to quantify biokinetic (BK) parameters was by fitting the integrated form of bioaccumulation models (Eq. 1) to measured organ-specific metal accumulation profiles for constant water exposure by using iterative nonlinear regression. ([30][17]),

$$C(t) = C(0)e^{-(k_{ew}+k_{ef}+k_g)t} + \left[\frac{(k_{uw}C_w)+(k_{uf}C_f)}{(k_{ew}+k_{ef}+k_g)}\right]\left(1-e^{-(k_{ew}+k_{ef}+k_g)t}\right), \quad (2)$$

where $C(0)$ is the initial concentration of the metal in the organ prior to the trial. Generally, for the combined uptake-elimination rate constant-based formulation, BK parameters could be estimated simultaneously from linear phase ([24][13]) or from the period that organisms are allowed to accumulation until internal metal concentration approach practical steady-state concentration of experiments (i.e., 95% steady-state concentration or 70% time period for the steady-state concentration) ([30]). The dietary BK parameters (k_{uf} and k_{ef}) was firstly determined by fitting Eq. (2) to measured data from dietary exposure assays by assuming C_w to be 0 μg mL^{-1} and the rates of metal uptake and elimination from water (i.e., k_{uw} and k_{ew}) could be ignored. Concentration-dependent waterborne BK parameters (k_{uw} and k_{ew}) were estimated from the observed data of dietary and water-borne exposure assays with the input of dietary BK parameters. Organ-specific bioconcentration factor (BCF) or biomagnifications factor (BMF) can be calculated as: BCF (or BMF) = (k_{uw} or k_{uf})/(k_{ew} or k_{ef}+k_g), representing the net accumulation ability that is the result of the competition between uptake, elimination from both waterborne or foodborne sources and growth dilution.

The relative importance of waterborne and dietary Cu fro organ residues could be calculated as the ratio of $(k_{uw}\times C_w)/(k_{uw}\times C_w + k_{uf}\times C_f)$ and $(k_{uf}\times C_f)/(k_{uw}\times C_w + k_{uf}\times C_f)$, respectively ([31]). We employed a steady-state bioaccumulation model approached ([24]) to understand how the tilapia cope the accumulated Cu through internal transition between organs when exposed to 0.6 μg mL^{-1} Cu. We assumed that all of Cu depurated from the gills, alimentary canal, and muscle were moved into the circulatory systems of tilapia and finally ends up in the liver. Accordingly, the steady-state Cu concentration in the liver could be predicted as

$$C_{ss}^{liver}(t) = \frac{\left(k_{ew}^{gill}\times C_{ss}^{gill}\right)+\left(k_{ew}^{AC}\times C_{ss}^{AC}\right)+\left[\left(k_{ef}^{MS}+k_{ew}^{MS}\right)\times C_{ss}^{MS}\right]}{\left[k_{ew}^{gill}+k_{ew}^{AC}+\left(k_{ef}^{MS}+k_{ew}^{MS}\right)\right]}, \quad (3)$$

where C_{ss}^{liver} is the steady-state liver Cu residue, C_{ss}^{gill} is the steady-state Cu concentration in the gills, C_{ss}^{AC} is the steady-state Cu concentration in the alimentary canal, C_{ss}^{MS} is the steady-state muscle Cu concentration, respectively.

A standard analysis of variance test (one-way or two-way ANOVA) was used to determine the significance of differences between the data set of Cu accumulation profiles between treatment groups and control. Student's *t*-test was carried out to compare metal residues between sampling times (corresponding to day 0 of the bioassay). This study employed the nonlinear option of the Statistica® software (StatSoft, Tulsa, OK, USA) to perform all curve fittings in this study and to calculate the coefficient of determination (r^2) and statistical analyses (ANOVA and Student's *t*-test). Statistical significance is judged when *p* values are less than 0.05.

III. RESULTS

A. Relative contribution of waterborne and dietary Cu

For the tilapia exposed to dietary Cu, the highest Cu residues were measured in the liver, followed by the alimentary canal and muscle (Fig. 1). Dietary Cu was also detected in the gills, suggesting that internal Cu residue transfers occurred via the circulatory system between organs. The mean uptake and elimination rates from dietary Cu exposures (i.e., k_{uf} and k_{ef}) were estimated (Table I). Values of k_{uf} were assumed to be proportional to the food consumption rates in case of appetite inhibition. Food consumption rates were recorded as 100% under treatments of 0.1 and 0.2 μg mL^{-1}, 40% and 27% in groups of 0.3 and 0.6 μg mL^{-1}. Results indicated that water was the main source of Cu for tilapia gills (>94%) and alimentary canal (>86%); food source Cu accounted for majority of metal residues in the muscle (> 51%) in treatments of 0.1, 0.3 and 0.6 μg mL^{-1}, and in the liver (60%) when exposed to 0.1 μg mL^{-1} Cu. The contribution of waterborne Cu to the liver Cu increased with the increasing of waterborne Cu concentration, however, did not changed obviously in other organs (Table II). Growth dilution was neglected in bioaccumulation models in this study because the measured growth rate in different treatments (ranged from -0.21 to 1.77%) was nearly two order lower than the values of k_e estimated in this study (Fig. 2, Tables I and II).

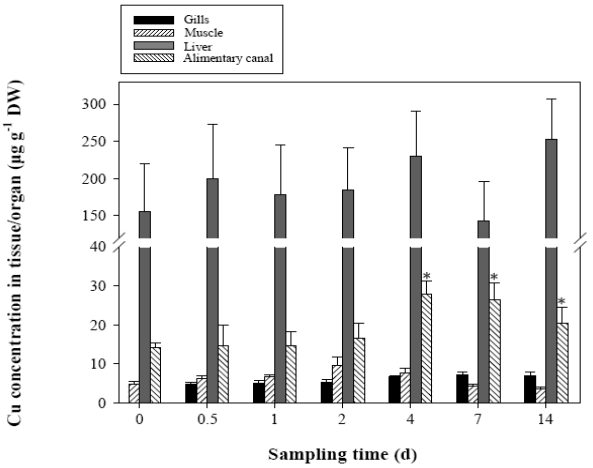


Fig. 1 Time series of Cu concentrations (Mean±SD, *n*=6) observed in tilapia organs during the 14-d dietary Cu exposures. Asterisks indicate significant difference of Cu residues in organs relative to the concentration at the beginning of bioassays

TABLE I

ESTIMATED VALUES (MEAN±SE) OF DIETARY CU UPTAKE RATE (k_{uf}), DEPURTION RATE (k_{ef}), AND BIOMAGNIFICATION FACTOR (BMF) IN SELECTED TISSUES OR ORGANS OF TILAPIA EXPOSED TO CU-SPIKED FOOD (13.5 ± 0.51 μg g^{-1}) FOR 14 DAYS.

Organ	k_{uf} (μg g^{-1} d^{-1})	k_{ef} (d^{-1})	BMF (μg g^{-1}) [a]	r^{2b}
Gills	0.07±0.04	0.12±0.08	0.58	**0.92***
Muscle	0.94±1.25	1.75±2.41	0.53	0.81
Liver	3.36±2.41	0.17±0.13	19.31	0.76
Alimentary canal	0.46±0.48	0.25±0.29	1.84	0.65

[a] Organ-specific biomagnification factor (BMF) can be calculated as: BMF=k_{uf}/k_{ef}.
[b] *p<0.05.

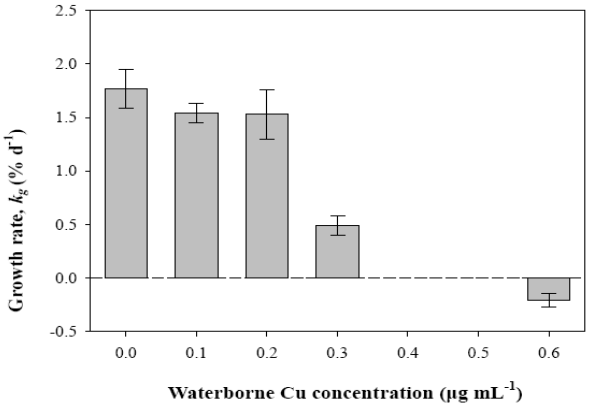


Fig. 2 Averaged growth rate (Mean±SD, n=6) of freshwater tilapia *O. mossambicus* exposed to assigned waterborne Cu concentrations for 14 days

B. Organ-specific waterborne Cu bioaccumulation and fits of biokinetic model

Result revealed a general pattern of Cu accumulated quickly and reached steady-state concentrations during the first few days of both waterborne and dietary exposures (Figs. 3-5). The maximum level of Cu residues was observed in the liver, following by alimentary canal, gills, and muscle, respectively, in the end of experiments.

There were no significant differences in the time course of Cu residues between treatments when the waterborne Cu (C_w)≦0.3 μg mL^{-1}. The temporal trend of the gills, alimentary canal and muscle Cu accumulation curves showed single peak accumulation when C_w ≦0.3 μg mL^{-1}. Cu resides increased to peak concentrations within 1 to 7 days of the exposure and then showed a decreasing with time thereafter (Figs. 3-5). It showed that the tilapia were able to mediate Cu residues when the Cu accumulated exceeding a certain level. Substantial differences were apparent in biokinetic profiles of Cu in the liver. Liver Cu increased monotonously with time in each concentration and no reduces of the Cu content observed during bioassays (Fig. 6).

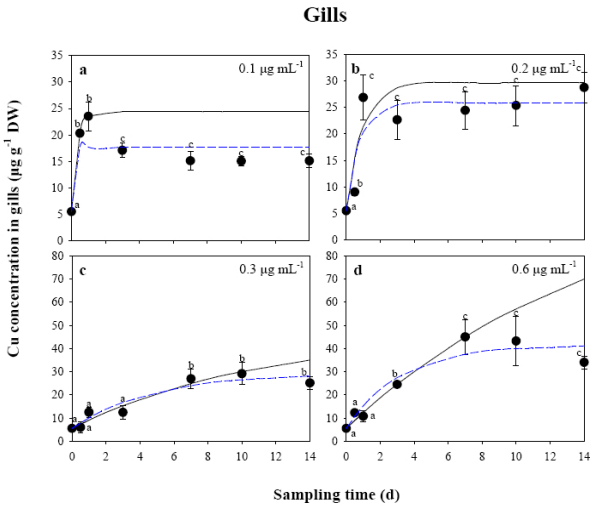


Fig. 3 Time series of Cu accumulation (Mean±SD, n=6) in the gills of tilapia exposed to dietary Cu (13.5 μg g^{-1}) and selected waterborne Cu concentrations for 14 days. Fitted lines show the comparison between the biokinetic model predictions for linear phase (solid line) and for the entire exposure duration (dash line). Results with different letters are statistically different from each other (ANOVA; Tukey's HSD, p<0.05)

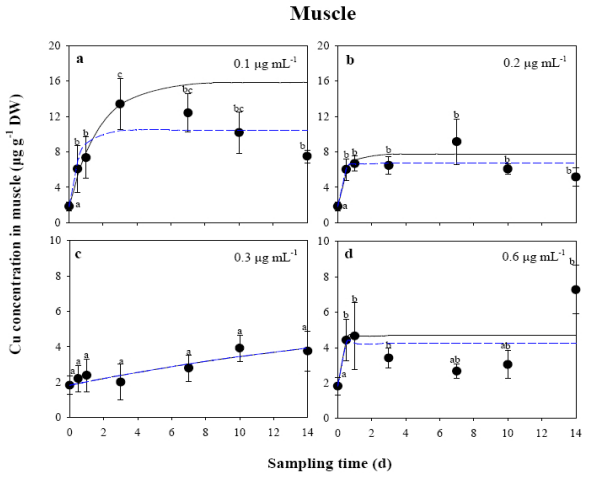


Fig. 4 Time series of Cu accumulation (Mean±SD, n=6) in the muscle of tilapia exposed to dietary Cu (13.5 μg g^{-1}) and selected waterborne Cu concentrations for 14 days. Fitted lines show the comparison between the biokinetic model predictions for linear phase (solid line) and for the entire exposure duration (dash line). Results with different letters are statistically different from each other (ANOVA; Tukey's HSD, p<0.05)

Similar to the gills, no significant difference in the time course of Cu residues between treatments were observed in the liver (p<0.05). Results indicated that the mediation for liver Cu was relatively insensitive comparing to these in muscle, gills, kidney, and alimentary canal once the accumulated Cu exceeded a certain threshold. In general, Cu residues in muscle tissue of tilapia are considerably lower (p<0.05) than those in other tissues/organs (Fig. 4).

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TABLE II
RELATIVE CONTRIBUTION (%) OF WATER AND FOOD AS SOURCES OF CU FOR SELECTED ORGANS OF TILAPIA EXPOSED TO VARIOUS WATERBORNE CU CONCENTRATION FOR 14 DAYS.

	0.1 μg mL^{-1}		0.2 μg mL^{-1}		0.3 μg mL^{-1}		0.6 μg mL^{-1}	
Organ	water	food [a]	water	food [a]	water	food [a]	water	food [a]
Gills	95	5	97	3	94	6	98	2
Muscle	49	51	80	20	45	55	40	60
Liver	40	60	89	11	97	3	94	6
Alimentary canal	95	5	94	6	86	14	98	2

[a] Cu concentration in food is 13.5 ± 0.51 μg g^{-1}.

TABLE III
COMPARISON BETWEEN ESTIMATED VALUES (MEAN±SE) OF WATERBORNE CU UPTAKE RATE (K_{UW}), DEPURATION RATE (K_{EW}) AND BIOCONCENTRATION FACTOR (BCF) IN SELECTED ORGANS OF TILAPIA IN VARIOUS WATERBORNE LEVELS FOR ENTIRE 14-D EXPOSURE DURATIONS AND LINEAR ACCUMULATION PHASES. FISH WERE EXPOSED TO CU-SPIKED FOOD(13.5 μG^{-1}) AND WATERBORNE CU SOLUTIONS SIMULTANEOUSLY

		Linear phase					Entire exposure				
Organ/treatment (μg mL^{-1})		Fitted days	k_{uw} (mL g^{-1} d^{-1})	k_{ew} (d^{-1})	BCF[a]	r^2 [b]	Fitted days	k_{uw} (mL g^{-1} d^{-1})	k_{ew} (d^{-1})	BCF[a]	r^2 [b]
Gill/	0.1	0-1	746.2±0.7	3.1±0.03	241	**0.99****	0-14	176.5±4998	27.1±3681	7	0.67
	0.2	0-1	158.3±87.5	1.1±0.7	144	**0.80***	0-14	167.9±77.8	1.3±0.6	129	0.80
	0.3	0-14	14.2±5.4	0.1±0.08	155	**0.94***	0-14	20.7±7.4	0.2±0.1	103	**0.91****
	0.6	0-4	12.3±2.5	0.06±0.05	205	**0.99****	0-14	21.6±7.4	0.2±0.1	108	**0.91****
Muscle/	0.1	0-2	90.5±15.2	0.6±0.1	151	**0.99***	0-14	133.3±66.4	1.2±0.6	111	0.76
	0.2	0-7	263.6±272.1	3.9±4.2	66	0.86	0-14	83.0±40.0	2.2±1.2	38	0.69
	0.3	0-7	0.9±0.8	0.04±0.09	23	0.84	0-14	0.9±0.8	0.04±0.09	23	0.84
	0.6	0-1	3.8±2.9	0.3±0.2	13	**0.97****	0-14	37.3±0.7	4.8±0.01	8	0.49
Liver/	0.1	0-14	7123.0±842.9	1.3±0.89	5479	**0.73****	0-14	7123.0±842.9	1.3±0.89	5479	**0.73****
	0.2	0-14	2953.6±446.6	0.4±0.35	7383	**0.67***	0-14	2953.6±446.6	0.4±0.35	7383	**0.67***
	0.3	0-14	1124.1±257.1	0.4±0.11	3389	**0.95****	0-14	1124.1±257.1	0.4±0.11	3389	**0.95****
	0.6	0-14	303.6±70.5	0.2±0.06	1515	**0.95****	0-14	303.6±70.5	0.2±0.06	1515	**0.95****
Alimentary canal/	0.1	0-2	2091.2±204.0	1.2±0.4	1742	**0.92***	0-14	1278.9±204.0	2.1±.76	609	0.49
	0.2	0-4	309.3±20.6	0.1±0.02	3093	**0.99****	0-14	472.9±128.4	0.3±0.01	1573	**0.94****
	0.3	0-14	50.2±32.6	0.06±0.1	836	0.83	0-14	50.1±32.6	0.1±0.06	836	0.83
	0.6	0-2	101.6±21.2	0.2±0.1	505	**0.99***	0-14	184.7±18.5	0.8±0.5	230	**0.73****

[a] Organ-specific bioconcentration factor (BCF) can be calculated as: BCF=k_{uw}/k_{ew} (mL g^{-1}).
[b] *p<0.05,**p<0.01,***p<0.001.

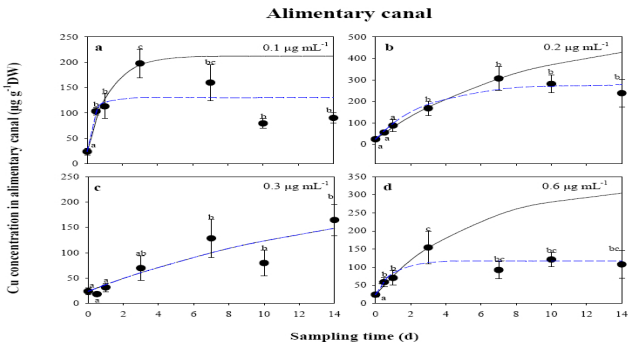


Fig. 5 Time series of Cu accumulation (Mean±SD, n=6) in the alimentary canal of tilapia exposed to dietary Cu (13.5 μg g^{-1}) and selected waterborne Cu concentrations for 14 days. Fitted lines show the comparison between the biokinetic model predictions for linear phase (solid line) and for the entire exposure duration (dash line). Results with different letters are statistically different from each other (ANOVA; Tukey's HSD, p<0.05)

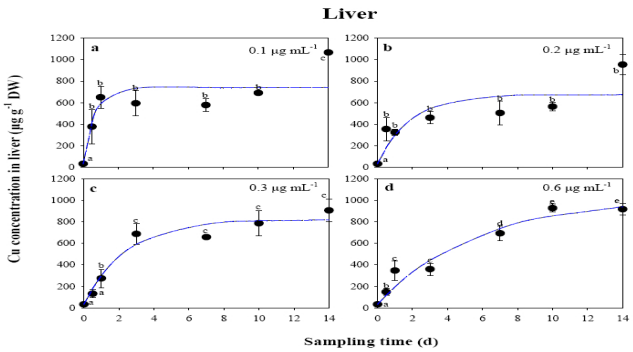


Fig. 6 Time series of Cu accumulation (Mean±SD, n=6) in the liver of tilapia exposed to dietary Cu (13.5 μg g^{-1}) and selected waterborne Cu concentrations for 14 days. Fitted lines show the comparison between the biokinetic model predictions for linear phase (solid line) and for the entire exposure duration (dash line). Results with different letters are statistically different from each other (ANOVA; Tukey's HSD, p<0.05)

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Figures 3-6 shows the comparison between the fitting curves of the biokinetic model to linear phases of Cu accumulation profiles and data from the entire experiments in selected tissues/organs. We found that the model well fitted all of the experimental data in every organs for the linear phases (r^2 = 0.67 to 0.99, Table 3). On the contrary, despite the model fitted the data well for the entire liver data, however, model predictions deviated from the observed data when Cu residues exceeding a certain level in the gills, alimentary canal, and muscle (Figs. 3-5). From the linear uptake phases, values of organ-specific k_{uw} ranged between 0.9 and 7123.0 mL g^{-1} d^{-1}. The highest k_{uw} occurred in the liver and followed by alimentary canal, gills, and muscle tissue (Table III).

BCF values of target organs were all above 1 (range from 13 to 7383) (Table III), suggesting the potential to accumulate Cu. Liver was characterized as the organ with the highest BCF value and steady-state Cu concentration among tissues/organs (Fig. 6). Despite the muscle account for over 70% of the total fish biomass, yet it had the lowest ability to accumulate Cu. Results showed that the uptake rate constant (k_{uw}), elimination rate constant (k_{ew}), and bioconcentration factor (BCF) among all of the selected fish tissues/organs obviously also depended upon the exposure concentration and exposure duration. In general, k_{uw}, k_{ew}, and BCF, decreased with the increasing of waterborne metal concentration (Table III). The drastically decrease in k_{uw} accounted for the corresponding decreasing in BCF. The extension of exposure duration also leaded to the change in the process of Cu biokinetics. We found that increase in k_{ew} responsed for the decrease of their BCF with time.

There was no general pattern observed in the change in k_{uw} of organs. In addition, there was no positive correlation found between Cu residues and waterborne Cu levels, which also suggested that the fish regulated actively Cu accumulation in their tissues/organs during prolong exposures (Figs. 3-6).

The relatively higher r^2 values of model fits in liver also suggested that the liver Cu residues is only slightly mediated by changing biokinetic processes in near sublethal exposures (e.g., 0.6 μg mL^{-1}, Table III and Fig. 6). Results indicated that the uptake rate and depurate rate constants for the liver are relatively insensitive to changes in liver Cu concentration, comparing to the cases in the gills, alimentary canal and muscle. Results revealed that assuming constancy of biokinetic processes could lead to incorrect predictions limitation by overestimating the Cu residues for chronic Cu exposures.

C. Biokinetic coping mechanisms

The total Cu accumulation in whole lumped sample increase as the waterborne Cu concentration increased (Fig. 7).

In terms of Cu burden, over 46.2%-56.8% of the total Cu burden of tilapia was recorded in the liver and followed by the alimentary canal (13.4-32.0%) and muscle (14.6-26.7%), at the end of the 14-d Cu exposures (Fig. 7), respectively. The amount of Cu continues to increase in the liver, however, the time trend of Cu burdens decreased or fluctuated in other organs/tissues (e.g., the gills, alimentary canal, and muscle) with the progress of duration. The liver played an important role in accumulation of Cu with the increase of duration, however, the contribution of muscle decrease with time. Muscle played a key role in

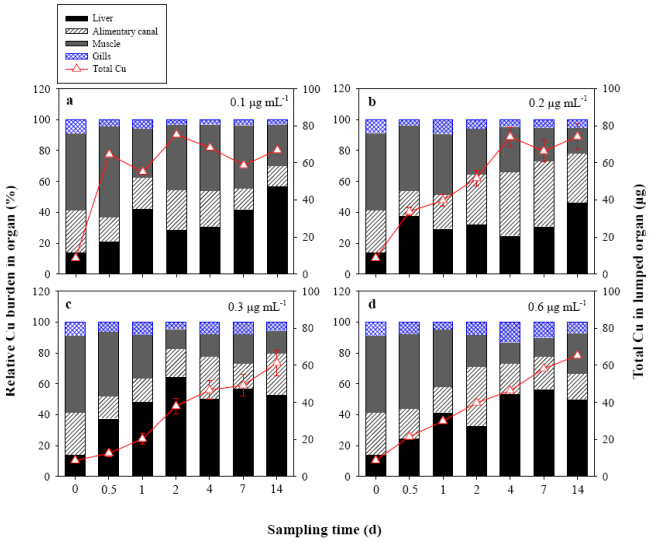


Fig. 7 Temporal trends of relative Cu burdens (%) observed in selected organs of tilapia and the corresponding total amount of Cu in the whole lumped organs during the 14-d exposure periods in various waterborne Cu treatments

buffering the rapid accumulation of Cu in the initial period of exposures, alternatively, the liver acted a more important role in the storage of Cu with the extension of exposures. Although the proportions of Cu were somewhat variable in the gills and alimentary canal, however, this variation seems not related to the treatment levels and durations. We found that the physiological mediation for mitigating the Cu accumulation including not only decrease the metal uptake or enhanced the depuration but also involved accelerated movement of Cu into the liver for storage via the circulatory system.

With the input of the steady-state Cu concentration in the gills (C_{ss}^{gill}, 938.6 μg g^{-1}, Fig. 3), alimentary canal (C_{ss}^{AC}, 0.8 μg g^{-1}, Fig. 5), muscle (C_{ss}^{MS}, 4.3 μg g^{-1}, Fig. 4), and organ-specific depuration rates (k_e) (Table 3) into Eq. (3), the steady-state liver Cu concentration was calculated as 17.8 μg g^{-1}, which is about 53 times lower than the actual value (938.6 μg g^{-1}). Result implied that the increase in liver Cu burden could account for all of the Cu lost from the gills, alimentary canal, and muscle.

The storage in or elimination via the liver is more critical than directly depurated to external environment through two surface organs (i.e., the gills and alimentary canal) or absorbed by rest part of fish body.

IV. Discussion

A. Changes in biokinetic rates during chronic exposures

Our study revealed that the biokinetic process of Cu was highly concentration- and time-dependent, suggesting that Cu residues were under active physiological controls or saturation occurred in these organs. This seems to be controversy to the basic assumption of the classic bioaccumulation model, which assumed that uptake and elimination rates, and bioavailability of chemical during exposure durations are unchanged over time.

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As the experimental Cu-spiked solutions were renewed regularly, the decrease in Cu residues in the gills, muscle and alimentary canal could be attributed to active physiological homeostasis processes, rather than the decrease in the bioavailable fraction of free Cu ions in water. Although there is currently no comparable study addressed how tilapia governing the Cu accumulation in the circumstance analogous to our study. However, studies related to Cu regulations in tissues/organs of freshwater teleost supported our findings. Carriquiriborde and Ronco ([32]) indicated that the value of bioconcentration factor of the gills and liver of pejerrey (*O. bonariensis*) showed an inverse relationship with waterborne Cu concentration when the fish were exposed to 50 and 100 μg L^{-1} Cu for 16 days. McGeer et al. ([33]) showed that biphasic profiles of Cu accumulation was observed in the gills, liver, and kidney of rainbow trout due to an active regulation for uptake and elimination of Cu when the fish were exposed to waterborne Cu (0.075 μg L^{-1}) up for 65 or 100 days. Similar patterns were also observed in selected organs of European eel ([12][4]). However, quantitative models for the relationship between the rates of uptake and elimination, exposure concentration, and exposure duration were absence in these studies. We found that the liver was highly responsible for the total Cu accumulation and it is the only organ did not downregulate its ability to accumulate Cu. Liver is reported as a primary site for the storage of Cu due to the sequestering of Cu by the metallothioneins before the elimination of excess Cu via bile ([34][35]). For the fish that acclimated for Cu exposures, they could store and metabolize the accumulated Cu as inactive form in the liver by inducting the metallothionein that increase the higher turnover rate of hepatic Cu for bile excretion. However, once the liver Cu residue exceeded a threshold, the homeostatic control for Cu became failed and then the Cu residue can increase ([4][36]). Our findings were consistent with to these studies because we found that the liver Cu concentration reached a plateau of accumulation at around 350 μg g^{-1} dw in day 2 or 3 after the start of the exposure in treatments of 0.1, 0.2, and 0.3 μg mL^{-1}. We found that two interface organs (i.e., the gills and alimentary canal) and muscle tissue showed a relatively higher regulatory ability to modulate the internal Cu content compared to the metal storage organ (i.e., the liver). Our finding was consistent with the pattern observed in Grosell et al. ([4]), McGeer et al. ([33]), Kraemer et al. ([14]), and Carriquiriborde and Ronco ([32]). Their studies revealed a rapid loading of Cu into these two organs, followed by a partial clearance then the Cu residue reached lower steady-state concentration. Because the gills and guts of fish are the organs that contact with external environment directly and they are capable to alter their ability to uptake and (or) eliminate of waterborne metal in order to mitigate possible toxicity induced by exceeding metal residues. Fish might be able to accelerate Cu elimination from the gills and alimentary canal either by transferring Cu to other organs or by excreting Cu to external environment when Cu accumulation exceeds a critical level, resulting in fluctuating residue trajectories of Cu residues. We found that alimentary canal accumulated appreciable amounts of Cu from aqueous exposures, because it showed high ability to accumulate waterborne Cu and accounted for large portion of the Cu accumulation in the whole body (from 13.4 to 32.0% of the total accumulated Cu). Similar pattern was also observed, but less distinct, when tilapia were exposed to 0.1 μg mL^{-1} Cu ([37]). Because the freshwater fish do not drink appreciably and the contribution of diet uptake rout was minor in this study. Thus the metal accumulated by alimentary canal should derived from aqueous source via gill respirations and (or) ion exchanges, and internal circulation. Pelgrom et al. ([37]) suggested that Cu accumulation in the alimentary canal might be a specific mechanism for tilapia, because, in general, about 95% of the whole body Cu was allocated into the liver for other exposed fish species. A sufficient accumulation and high depuration rate via alimentary canal might be response for the higher tolerance of tilapia to metal toxicity comparing to other species.

B. Transition of Cu between organs

We illustrated the trend of the Cu distribution between organs in different treatments in order to understand how the tilapia mediate the Cu residues. The total Cu burden increased with the extension of the exposure duration and accompanied with decreased Cu level in the muscle and increased in the liver, indicating interchanges of Cu between tissues or organs, rather than the just loss to environment. The relative amount of total Cu continuously increased in the liver, in contrast, Cu accumulation in muscle, gills and alimentary canal tended to plateau or descent, suggesting that the biokinetic mediation in tilapia including accelerating the movement of gill and muscle Cu into the liver via circulatory system rather than just suppressed the Cu uptake or accelerates the elimination across the apical epithelial membrane into the external environment.

Overall, the organ-specific Cu distribution profiles revealed two main strategies of Cu mediation in tilapia. Firstly, although the Cu concentration in muscle tissue is relative lower, comparing to other organs (about 10 to 30 times lower than alimentary canal and liver, respectively), nonetheless, around 50% of the total Cu residue accumulated in the muscle during the initial period of the exposure because it accounted over 80% of the total fish biomass. The proportion of Cu in muscle rapidly decreased with the extended of the duration in each treatment. This suggested that muscle tissue plays key role for short-term Cu storage. In other words, muscle tissue serves as a buffer or surge protector. Secondly, contrary to the muscle working as a metal buffer in short-term exposures, the liver acting importantly in scavenging Cu toxicity with the extension of exposure. The increase of Cu in the liver with the extend of duration could be attribute to its high net metal bioaccumulation ability for Cu in steady-state conditions (BCF = 1515-7383 mL g^{-1}).

To our knowledge, this is the first study addressing how the internal transition of Cu across the time course and treatments between organs of tilapia. Our data showed a similar trend with the finding for Ag accumulation in two freshwater teleosts (rainbow trout and European eel) through water ([38]). The contribution of the liver and gut Ag increased from 25% after 1 day to 70-80% after 67 day exposures, because fish have more time to re-distribute newly accumulated Cu from muscle tissues to the liver or alimentary canal for excretion to minimize toxic effects.

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C. Model application to organ-specific bioaccumulation

Under these simulation conditions, dissolved Cu and dietary Cu are both possible source of Cu to tilapia. The biokinetic model tended to overestimate the Cu concentrations in the gills, muscle and alimentary canals, notably in extending exposure periods. Because the biokinetic model does not currently consider possible protection of biokinetic regulations of fish (i.e., changes in the process of k_u, k_e and BCF) as well as changes in fish feeding behaviors (including feeding rate and assimilation rate), and other factors that might influence accumulation efficiency in the fish. Poor modeling for metal accumulation in freshwater fish were also observed in other studies, for example, obvious changes in Cd accumulation processes in wild yellow perch, *Perca flavescens* ([24]). Yellow perch reduced the Cd concentration in the gills and guts once the metal residues reached a certain level in these organs. Similar to our case, the good agree between modeled and observed data was only observed in liver metal concentration. Bioaccumulation models generally overestimated steady-state Cd concentrations because *P. flavescens* might be able to moderate accumulation by altering their ability to take up and (or) depurate through the gills and guts. Results supports that the classic biokinetic model was poor in explicitly illustrating the metal residues especially in case of prolonged exposures. Results implied that the extrapolating of metal residues between different treatments with biokinetic rate constant estimated from single treatment is also questionable, because the uptake and depuration rate constants showed dependence not only on exposure duration and but also on waterborne metal concentration. Luoma and Rainbow ([22]) suggested that the assumption of a constant uptake rate may merely be acceptable in the natural condition or even up to concentrations that are an order of magnitude higher than those see in nature. The uptake rate could be subjected to saturation kinetics and competitive inhibition between ions at very high concentrations, because most of the metal ions pass through the gill membranes via channels or carriers and other facilitated diffusion processes (e.g., the carrier molecule like membrane-bound ATPase) ([30]). The assigned Cu concentration in our experiments, were up to 1 or 2 orders higher than these in field ecosystems, thus the reduction of uptake rate constant would be acceptable. This may explain why the k_u decrease with time course of exposure and of waterborne Cu concentration.

A first-order bioaccumulation model was developed for the change in bioaccumulation of organic contaminate over the time course of exposures as $C_i(t) = C_i(0)e^{-k_{ew}t} + \frac{k_{uw} \times C_w}{k_{ew} - \lambda} \times \left(e^{-\lambda t} - e^{-k_{ew}t}\right)$, where λ refers to the rate constant (d^{-1}) that allows for decreasing levels of chemical over the course of the exposure. This model ascribed the biokinetic change to the difference in bioavailability and/or the potential transformation of chemical within organisms ([39][40]). Although this model has not been validated for metal accumulations, the latter process is certainly a possibility because subcellular Cu partitions and metallothionein concentrations in fish tissues were revealed to be a primary role in Cu regulation during long-term exposures in marine and freshwater fish ([14][13][41]).

In summary, our finding indicated that the bioaccumulation processes of Cu in tilapia were highly dependent on exposure durations and concentrations. This is strongly against the basic assumption of the classic bioaccumulation model.

We suggested that the classic bioaccumulation model is only validated in specific organs (e.g., the liver) which do not obviously change in their biokinetic behaviors during extending exposures or between various treatments. Considerations of changes in metal bioaccumulation behavior by active biokinetic coping mechanisms are necessary when modeling Cu accumulations in the gills, alimentary canal, and muscle tissues, especially for the chronic ecotoxicological risk predictions.

Future models are suggested to integrate organ-specific biokinetic mediation with the knowledge of subcellular participation of metal for predicting the active Cu dose in specific organs.

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