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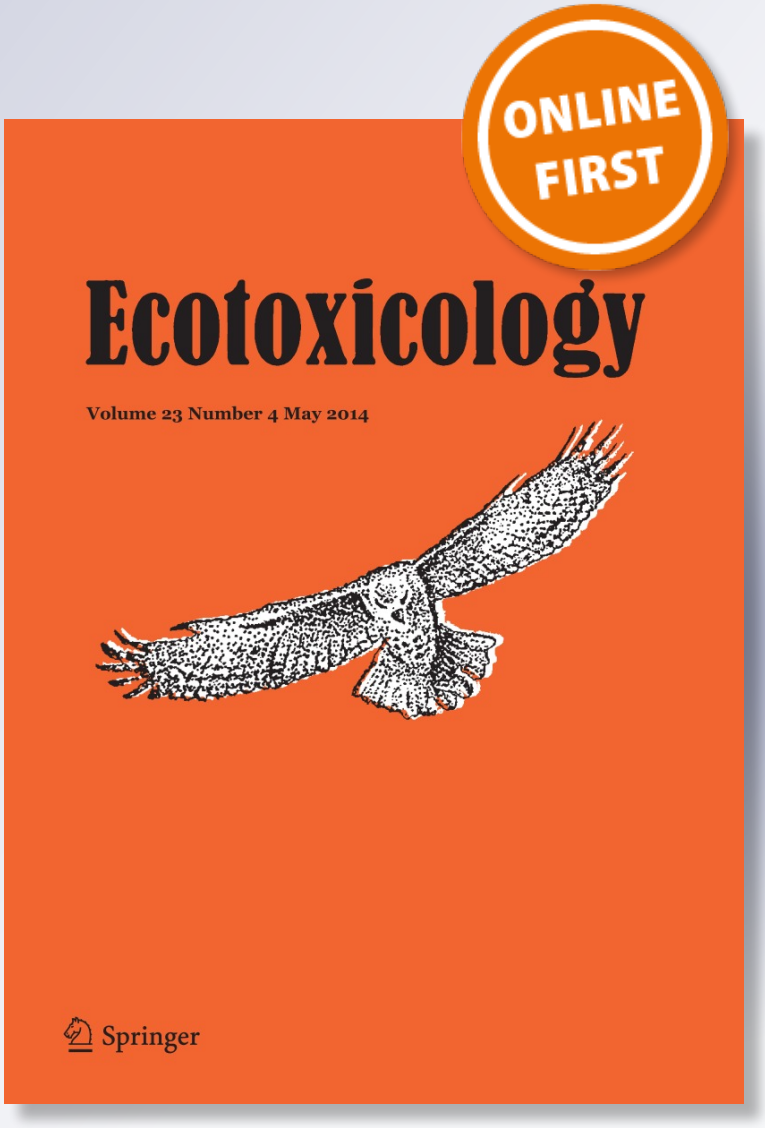

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Interpreting copper bioaccumulation dynamics in tilapia using systems-level explorations of pulsed acute/chronic exposures

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Abstract To understand how environmental variability could impose aquatic organisms in response to altered disturbance regimes and temporal patterns of waterborne toxicants is challenging. Few studies have reported in an organ/tissue specific basis, and most studies have been restricted to steady-state conditions. For interpreting systematically copper (Cu) bioaccumulation in tilapia (*Oreochromis mossambicus*) in a pulse scheme, we combined mechanistic and statistical as well as model-based data analyses of exposure data that cover short-term mortality to long-term organ/tissue growth bioassay. Our present pulsed Cu-tilapia physiologically-based pharmacokinetic model was capable of elucidating the Cu accumulation dynamics in tissues of tilapia under different pulsed exposure scenarios. Under acute and chronic pulsed exposures, our study found that (i) stomach and kidney had the highest uptake and elimination capacities, (ii) liver was prone to a highest BCF and was more sensitive than the other tissues, and (iii) Cu accumulations in most of organs and other tissues were strongly dependent on the exposure pulse characteristics such as frequency and duration and not on concentration (i.e., amplitude). We showed that interactions across multiple pulsed or fluctuating Cu exposures were involved in accumulation changes that could also be achieved by controlling pulse timing and duration. The analytical approach we described provides an opportunity to examine and quantify metal accumulation dynamics for fish in response to environmental variability-induced non-uniform metal exposures on an organ/tissue-dependent scale and to integrate qualitative information with toxicokinetic and physiological data. We hope that our systems-level tools for mathematical analyses and modeling will facilitate future large-scale and dynamic systems biology studies in other model fish.

Keywords Copper · Bioaccumulation · Tilapia · Pulse dynamics · Ecotoxicology

Introduction

One of major challenges in ecotoxicology is to understanding changes in environmental variability that impose aquatic organisms in response to altered disturbance regimes and temporal patterns of waterborne toxicants. The underlying complexity arises from the intertwined nonlinear and dynamic interactions among a variety of pulse conditions. For example, when the degree of stochasticity of environments increases or extreme events become more frequent, such as the rainfall, accidental spillage of wastes, the periodic emission of anthropogenic contaminants can generate pulsed patterns. The diel metal cycles of biogeochemical process are greatly dynamics and short-term (daily and bihourly) variations in metal concentration (Authman and Abbas 2007; Tercier-waeber et al. 2009). The occurrence of diel variations in heavy metal concentrations were found in the surface water (Diamond et al. 2005; Gammons et al. 2007; Parker et al. 2007; Pereira et al. 2009, 2011; Heier et al. 2010).

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Copper (Cu) sulfate has been widely used as algaecide and molluscicide to exterminate phytoplankton and control parasite and skin lesion of fish in cultured ponds (Chen et al. 2006; Carvalho and Fernandes 2008). Cu plays an essential role in cellular metabolism and various physiological activities of aquatic organisms, yet high Cu level trend to induce toxic effect (Maiti and Banerjee 2000). However, acute and chronic stresses induced the different physiological challenges. De Boeck et al. (2007) demonstrated that Cu accumulates in the chloride cell and positively inhibit brachial Na^+/K^+-ATPase activities decreasing Na^+ transport in gill of fish, causing cardiovascular and mortality in fish. The chronic exposure, on the other hand, may lead to the organisms acclimated to the environment changes, increasing the tolerance to Cu stressor.

Authman and Abbas (2007) found a positive relationship between seasonal waterborne Cu concentration and organ-specific (gill, muscle, liver) concentration for tilapia (*Tilapia zillii*) in Lake Qarun, Egypt. Although Cu accumulative capacity in aquatic organisms has been widely investigated, most studies only focused on the particular organ/tissue to investigate the Cu transfer and detoxification (Pelgrom et al. 1995; Authman and Abbas 2007). Chen et al. (2012) indicated that Cu could induce histological alterations in gills and liver and altered Cu accumulation in several tissues including gills, liver, kidney, GI tract, and muscle in *Pelteobagrus fulvidraco*.

McGeer et al. (2003) found out that toxicokinetic data for Cu, Zn, Cd, Pb and other metals showed inverse relationship between bioconcentration factor (BCF) and aqueous exposure. Adams et al. (2003) further indicated that physiologically-based toxicokinetic mediation might be developed to overcome potential chemical stresses, especially during prolonged exposure to sublethal essential metal concentrations. Physiologically-based pharmacokinetic (PBPK) models have been widely used in several aquatic fish subjected to various chemicals, e.g., pyrene in rainbow trout (*Oncorhynchus mykiss*) (Law et al. 1991), cadmium in rainbow trout (*Salmo gairdneri*), dioxin in brook trout (*Salvelinus fontinalis*) (Nichols et al. 1998), 1,1,2,2-Tetrachloroethane, pentachloroethane, and hexachloroethane in lake trout (*Salvelinus namaycush*) (Lien et al. 2001), arsenic in tilapia (*Oreochromis mossambicus*) (Liao et al. 2005), and among others. However, they were performed at constant toxicant exposure scenarios and did not take the pulsed accumulation into account.

To better understanding the interactions between acute/chronic exposure schemes, the acquisitions of appropriate, preferably time-resolved quantitative data is a prerequisite. Because the acquisition of such data is technically demanding, few studies have reported in an organ/tissue specific basis, and most studies have been restricted to steady-state conditions (Reinert et al. 2002; Cogun and Kargin 2004; Kamunde and MacPhail 2008; De Boeck et al. 2010; Poleksic et al. 2010; Chen et al. 2012). To elucidate the dynamic interplay between pulsed acute/chronic exposures systematically, we investigated dynamic changes in Cu bioaccumulation in tilapia that cover short-term mortality to long-term organ/tissue growth bioassay. To date, we still have a much more limited understanding of the ecotoxicological consequences of changes in variability, and their potential interactions with other drivers of change. Particularly, there is a lack of experimental studies that truly tested for the consequences of changes in variability by explicitly manipulating environmental variability.

Therefore, the purpose of this study was combined mechanistic and statistical, as well as models-based data analyses of acute and chronic pulsed Cu accumulation to systematically understand the organ/tissue-dependent toxicokinetics and correlations among Cu burdens in different organs and tissues. Here we used tilapia (*O. mossanbicus*), the widely distributed in rivers and major human food sources in Taiwan, as an animal model to investigate the degree of transfer, detoxification, distribution dynamics, uptake, and elimination mechanisms under the pulsed acute and chronic Cu exposures.

Materials and methods

The experimental design, computational analysis, and information flow covered in this study were depicted in Fig. 1 and described in detail in the following sections.

Acute/chronic bioaccumulation bioassays

This study designed the short- and long-term pulsed Cu exposure experiments to examine the accumulation abilities in gills, blood, stomach, intestine, liver, kidney, gonad, muscle, bone, and carcass of tilapia *O. mossambicus*. Tilapia were 8-month old mature adult with mean length 10.88 ± 1.51 cm (mean ± SD) and mean body weight 19.69 ± 9.61 g wet wt. Fish were acclimatized for 14 days in the following conditions: water temperature 28 °C, pH 7.8, 12 h light cycle, dissolved oxygen (DO) 7.5 mg L^{-1}, and alkalinity 91.1 mg L^{-1}. The water chemistry characteristics were total Cu 0.004, Ca^{2+} 59.60, Mg^{2+} 13.17, Na^+ 9.40, K^+ 2.73, OH^- 0.013, and CO_3^{2-} 0.012 mg L^{-1}. Water source was tap water that has been dechlorinated before conducting the exposure experiments.

During the acclimation periods, fish were fed 1 day with commercial fish food. The exposure experiment was carried out with 90 adults under static conditions in six aquariums of 81 L volume (measuring 60 × 30 × 45 cm^{-3}) filled with 70.2 L of exposure

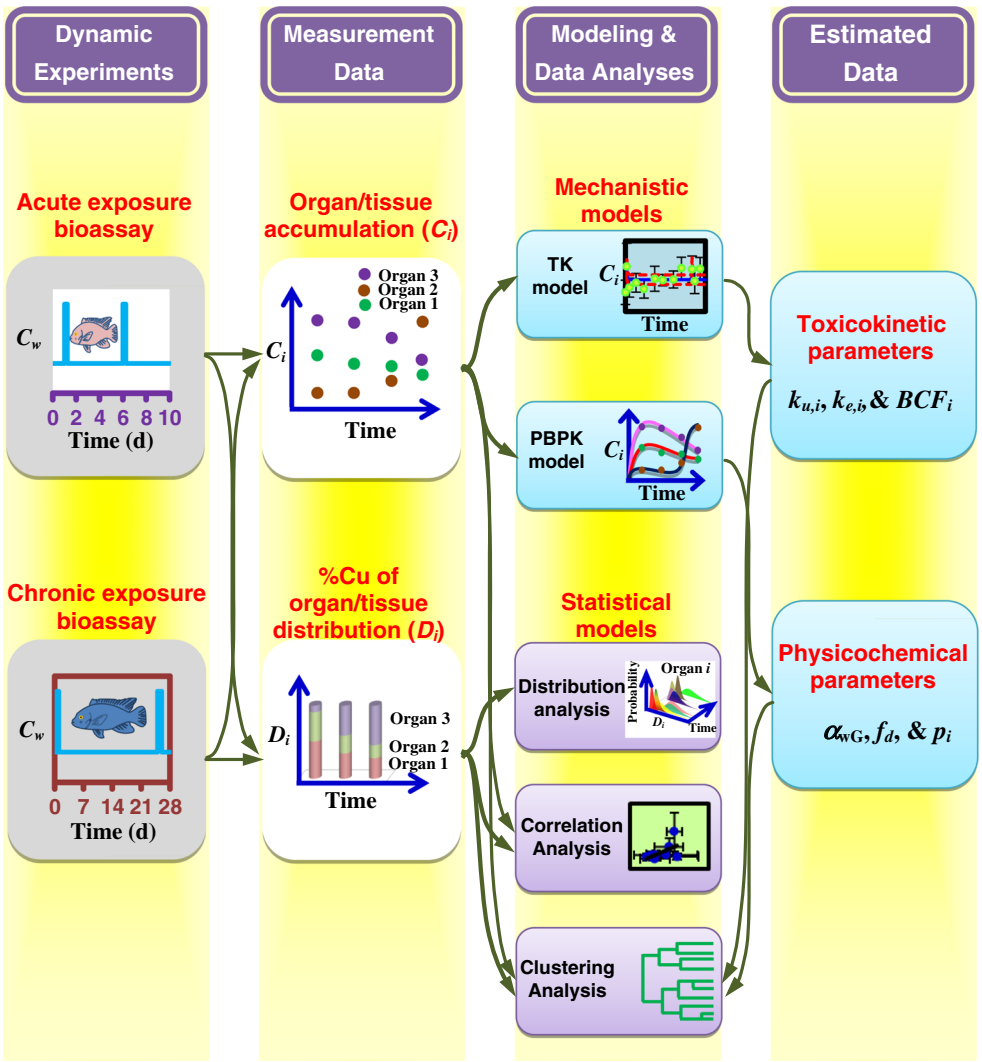

Fig. 1 Schematic showing experimental design, endpoint measurements, computational analysis, and information flow used in this study in that *arrows* show the flow of information

solution. Each aquarium was containing a stock density of 15 fish. The Cu sulfate ($CuSO_4 \cdot 5H_2O$) stock solution was prepared with double-deionized water. The sequential pulsed Cu exposure bioassays were carried out with short-term 10-day and long-term 28 day exposure periods exposed to pulsed Cu concentrations by increasing 1*X* to 3*X* μg L^{-1}, i.e., from 100 to 300 μg L^{-1}. The sequential pulsed Cu exposure design was accomplished by siphoning the volume of Cu contaminated water in the test aquarium from *X* L to 1/3*X* L (e.g., from 70.2 to 23.4 L), and filled water to *X* L in the test aquarium.

After water was siphoned from *X* L to 1/3 *X* L, Cu-amended water was filled to *X* L in the test aquarium to increase the pulsed concentration and vice verse. The pulsed Cu exposure timings were occurred twice during the exposure periods at days 1 and 6 for short-term exposure and at days 0.5 and 25 for long-term exposure, respectively. The pulsed exposure duration was carried out 6 and 24 h in each event for short-term and long-term exposure, respectively. The entire Cu solution was replaced and collected daily to avoid the regression of water quality. The feces were removed every 6 h. The forage debris was collected every 1 h after feeding in the aquarium. One fish was removed from each experimental tank on days 0, 1, 1.25, 2, 3, 6, 6.25, 7, 8, and 10 of short-term exposure.

On the other hand, those of long-term exposure were removed on days 0, 0.5, 1.5, 4, 7, 11, 14, 18, 21, 25, 26, and 28. Tested fish were anesthetized with benzocaine hydrochloride solution during the sampling. The dissected organ/tissue samples were cleaned with double–deionized water and freeze dried overnight, and then grounded to fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 250 mg portion of the powder were digested with 2 mL 65 % concentrated HNO_3 and 1 mL 30 % H_2O_2 overnight

at 95 °C. The 20 mL organ/tissue samples were stored at −4 °C in the dark until they were analyzed.

Physiologically based pharmacokinetic model

This study developed the Cu-tilapia PBPK model for acute/chronic pulsed Cu exposures in *O. mossambicus* (see Supplementary text). The essence of almost all PBPK models can be described by a state-space model based on the mass-balance as (Supplementary Fig. S1)

$$\frac{d\{C(t)\}}{dt} = [A]\{C(t)\} + [B]\{u(t)\}, \quad (1)$$

where $\{C(t)\}$ is the state variable vector describing Cu burden in each target organ (μg g^{-1}), $\{u(t)\}$ is the input vector of pulsed Cu in water (μg L^{-1}), $[A]$ is the state matrix describing the exchange rate between target organs/tissues (L $days^{-1}$), and $[B]$ is the constant input matrix describing the exchange rate into target organs/tissues (L $days^{-1}$). The detailed PBPK model equations are listed in Supplementary Table S1.

The physiological parameters used in the Cu-tilapia model include blood volume and organ/tissue weight that can be obtained from experimental data, and exchange rates between tissue and blood compartments as the fraction of cardiac output. The cardiac output (Q_C, L h^{-1}) was scaled to body weight using allometric equation as $Q_C = 2.06 \cdot BW^{0.75}$ where 2.06 is the parameter value for 1 kg fish that adopted from Nichols et al. (1998) and BW is the body weight (kg). All physiological parameters used in the PBPK modeling were given in Supplementary Table S2. To acquire the physicochemical parameters, this study assumed that gill sorption factor has a resemblance to gill BCF.

Due to the instantaneous waterborne Cu concentration varying with time, the time-dependent tissue partition coefficient ($p_i(t)$) can be obtained by calculating the area under the curve (AUC) of Cu in tissue/AUC of Cu in blood for a given specific tissue. The gill sorption factor, the fraction of Cu dissolved in blood, and organ/tissue-specific partition coefficients of physicochemical parameters can be calibrated to experimental data of pulsed Cu accumulation. The calibration of the PBPK model was assessed by using mean absolute percentage error (MAPE), computed from $\text{MAPE} = \frac{1}{N}\sum_{n=1}^{N}\frac{|C_{e,n} - C_{s,n}|}{C_{e,n}} \times 100\ \%$ where N denotes the number of experimental data, $C_{e,n}$ is the experimental data, and $C_{s,n}$ is the simulation result corresponding to data point n.

Linear regression and cluster analyses

This study used a linear regression analysis to identify the correlations between the Cu accumulations in specific organs/tissues for investigating the similarity across the organ correlations in acute and chronic pulsed exposures scenarios. We distinguished 55 and 77 groups, respectively, in acute and chronic exposures to perform the cluster analysis. Each tissue/organ can be separated by groups based on pulsed timing/duration.

Here we selected two pulses: (i) during pulse #1 (acute: at day 1.25; chronic: at day 1.5), after pulse #1 (acute: at day 3; chronic: at days 7, 14, and 21), and (ii) during pulse #2 (acute: at day 6.25; chronic: at day 26), before pulse #2 (acute: at day 6; chronic: at day 25), and after pulsed #2 periods (acute: at day 10; chronic: at day 28). The groups of during and after pulse #1, as well as, before, during, and after pulse #2, were classified based on the basis of similarities within experimental data and estimated parameters, including AUC of Cu accumulation in tissue, uptake/elimination rate constants, BCF, and time-dependent partition coefficient.

Chemical analysis

The flame atomic absorption spectrometer (Perkin Elmer AA-200, USA) were used to analyze Cu organ/tissue burdens and water quality. Analytical quality control of tissue sample was achieved by digesting and analyzing identical amounts of rehydrated (90 % H_2O_2) standard reference material (dogfish muscle, DORM-2; NRC-CNRC, Canada). The waterborne ions concentrations, such as total Cu, Ca^{2+}, Mg^{2+}, Na^+, and K^+, were analyzed followed the standard methods based on APHA (1998). Standard solutions of ions were used to establish standard curves (Merck, Darmstadt, Germany). The 15 mL water sample with 200 μL HNO_3 were digested 2–3 h at 95 °C, then the water characterizations were determined by Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer ELAN DRC ROMAN II, USA). All samples were analyzed in three times. The recovery rate was 94.6 ± 3.6 % and the levels of detection were 20 μg Cu L^{-1} for water sample and 20 μg Cu g^{-1} for tissue sample. The experimental materials used in the exposure experiment included glassware and plastic implements. All glassware and plastic materials were cleaned by immersion in 10 % Decon for 1 day, then in 25 % HNO_3 for 2 days and finally rinsed with ultrapure water and dried. All experimental processes were performed by ultraclean technique during the sample manipulations.

Data analysis

A first-order one-compartment toxicokinetic model can be used to estimate toxicokinetic parameters of uptake and elimination rate constants for each organ/tissue by fitting

the accumulation experiment data under the sequential pulsed Cu concentration

$$\frac{dC_i(t)}{dt} = k_{u,i}C_w(t) - k_{e,i}C_i(t), \quad (2)$$

where $C_i(t)$ is the time-dependent Cu burden in organ/tissue i, $k_{u,i}$ is the uptake rate constant of organ/tissue i (mL g^{-1} $days^{-1}$), $k_{e,i}$ is the elimination rate constant of organ/tissue i ($days^{-1}$), t is the exposure time (d), and $C_w(t)$ is the pulsed waterborne Cu concentration (μg mL^{-1}) that can be expressed as,

$$C_w(t) = C_0 + C_1[U(t-T_1) - U(t-T_2) + U(t-T_3) - U(t-T_4)], \quad (3)$$

where $U(t - T_j)$ is the unit step function, C_0 and C_1 represent the initial and pulsed concentrations (μg mL^{-1}), and T_j is the pulsed exposure at timing j (d).

To determine the time-dependent % Cu of organ/tissue in whole body of tilapia in acute/chronic pulsed scenarios, we analyzed the pulsed Cu accumulation data of organ/tissue and whole body and incorporated the specific organ/tissue weight and whole body weights

$$D_i(t) = \frac{C_i(t) \times BW_i}{C_{wb}(t) \times BW_{wb}} \times 100, \quad (4))$$

where $D_i(t)$ is the time-dependent % Cu distribution of organ/tissue i, C_{wb} is the Cu concentration in the whole body at time t (μg g^{-1}), and BW_i and BW_{wb} are the weights of organ/tissue i and whole body of tilapia (g), respectively.

We employed the TableCurve 2D (Version 5, AISN Software, Mapleton, OR, USA) and 3D (Version 4, AISN Software, Mapleton, OR) to optimal fit the experimental data to obtain optimal statistical models. The Berkeley Madonna Modeling and Analysis of Dynamic Systems (Version 8.3.9) was used to perform the Cu-tilapia PBPK simulations. The Crystal Ball® software (Version 2000.2, Decisionerring, Inc., Denver, Colorado, USA) was employed to implement Monte Carlo simulation to obtain the 2.5th- and 97.5th- percentiles as the 95 % confidence interval (CI) for all fitted models. The dynamic probability distributions of % Cu in organs were generated from the lognormal distribution of simulation outcomes. It showed that 10,000 iterations were sufficient to ensure the results. The cluster analysis of acute/chronic pulsed Cu-tissue and dendrograms were performed by using Statistica® software 6.0 in that the experimental data and estimated parameter were used as log_2-transform set. Clustering was calculated by Ward's minimum variance method. The dendrogram depicts the hierarchical clustering based on the Euclidean distances.

Results

Toxicokinetic parameters estimation

Our results showed that in the acute pulsed Cu exposure, most of organs/tissues experienced rapid accumulation fashions at initial exposure period except gills, muscle, carcass, and bone, whereas a substantially instantaneous Cu uptake was found in all organ/tissue in chronic pulsed Cu exposure (Fig. 2a, b; Supplementary Table S3). Our results also demonstrated that a positive association was found for the relationship between elimination and uptake capacities of acute ($r = 0.418$) and of chronic ($r = 0.756$) pulsed exposure. In whole body, for instance, we found a reasonable fitted to pulsed exposure data (acute: $r^2 = 0.75$, chronic: $r^2 = 0.54$), resulting in uptake rate constant (k_u) estimates of 0.478 and 0.139 mL g^{-1} h^{-1} and elimination rate constant (k_e) estimates of 0.002 and 0.001 h^{-1} for acute and chronic exposure, respectively (Table S3). On average, we found that most k_u and k_e of organ/tissue and whole body in acute exposure were higher than those in chronic exposure.

The BCFs of the organ/tissue were very high (logBCF > 1.5), ranging from 22.11–8,841.76 to 38.25–6,063 for acute and chronic pulsed Cu exposures, respectively (Fig. 2c; Table S3). Thus a potential high Cu accumulation capacity in tilapia was organ/tissue-dependent. Moreover, the results showed that most of the chronic organ/tissue BCFs were less than those of acute exposure except for blood, muscle, carcass, and bone. The maximum and minimum levels of pulsed BCF were found in liver and carcass in both acute and chronic pulsed Cu exposures with a decreasing order in acute exposure as intestine > kidney > gonad > stomach > gills > blood > bone > muscle. On the other hand, the decreasing order in chronic exposure was kidney > stomach > intestine > blood > gonad > gills > muscle > bone.

Time-dependent % Cu distribution in organ/tissue

Generally, total Cu accumulation in the tilapia increased as the time increased in both acute and chronic exposures even after exposure with pulse Cu (Fig. 3). In the acute exposure (Fig. 3a), we found Cu distributions in blood and stomach increased during the first and second pulsed periods, whereas Cu distribution in kidney decreased at two pulsed exposure periods but increased after pulsed exposure. However, the Cu distributions in muscle, bone, and carcass decreased with exposure time, whereas intestine and liver were increasing continuously (Fig. 3a).

In the chronic exposure bioassay, Cu distribution in blood and gills experienced a fluctuating trend with pulsed

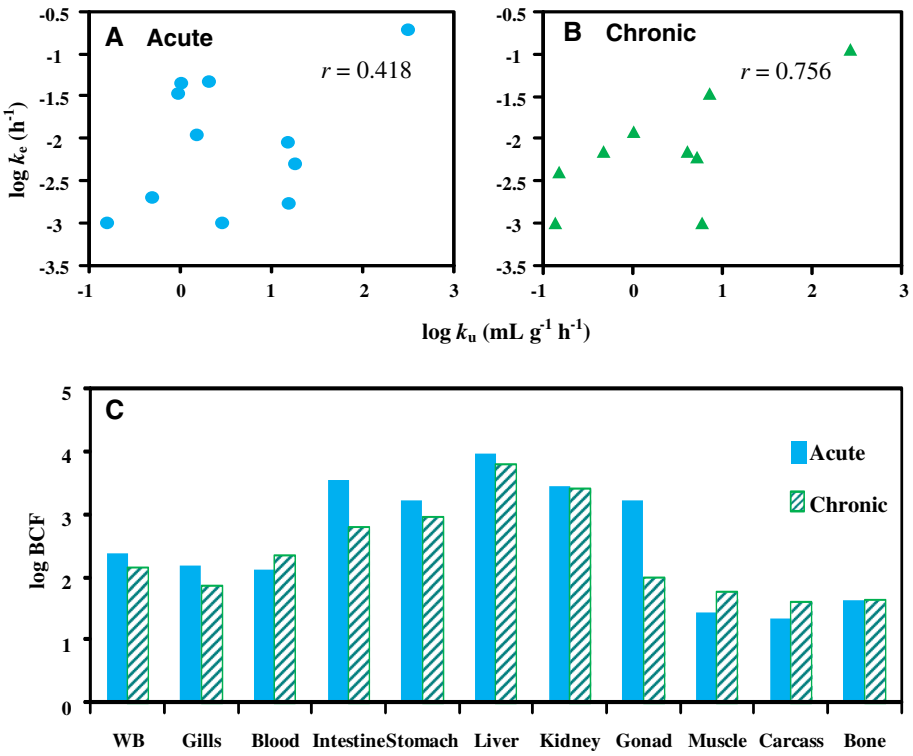

Fig. 2 Relationship between **a** acute and **b** chronic uptake (k_u) and elimination rates (k_e), and (C) BCFs in tilapia exposed to pulsed waterborne Cu

periods (Fig. 3b). We found the similar increasing trend of % Cu in liver and decreasing trends of muscle and bone compared with acute pulsed exposure bioassay (Fig. 3b). On the beginning of exposure, most Cu distribution was in muscle (37.14–50.38 %), followed by liver (14.13–32.04 %) and gills (10.06–10.13 %). However, at the end of acute and chronic exposure times, most Cu distribution was found in liver (51.75–56.53 %), followed by intestine (16.24–38.61 %) and muscle (10.61–15.30 %).

To identify the uncertainty in individual differences, we investigated the dynamic probability distribution of time-dependent % Cu in organ/tissue for all fish in each exposure scenario (Fig. 4; Supplementary Table S4). We found that blood and gill acted as the Cu transporters in both acute/chronic exposures, showing dynamic variations were not depend on external exposure pattern in that time-dependent % Cu distribution were not more than 1 % (geometric mean) for blood and 3–11 % for gills (Fig. 4a, b). The most high % Cu distribution were in bone (7.47 %), carcass (9.91 %), and muscle (49.35 %) occurred at initial acute exposure timing, whereas more than 75 % decrement (bone 1.10 %, carcass 2.49 %, and muscle 10.20 %) were appeared at the end of acute exposure bioassay (Fig. 4e, k, q).

On the other hand, % Cu in others organs/tissues (stomach, gonad, kidney, intestine, liver) at end of exposure time were more than those at initial exposure time (Fig. 4g, i, m, o, q). Notably, intestine and liver were experienced 1.5- to 2.5-folds higher. However, the results showed that kidney maintained uniform % Cu distribution ranged from 0.45 to 0.88 %.

The trends of most % Cu distribution in organs/tissues revealed the decrement at end of chronic pulsed exposure time (Fig. 4f, j, l, n, r) due in part to the most Cu accumulations were shifted towards stomach, intestine, and liver (Fig. 4f, p, t). However, % Cu distribution trends of intestine were not depend on both acute and chronic pulsed Cu exposures.

PBPK parameters estimation and model calibration

All of physicochemical parameters of acute and chronic pulsed exposures used in the PBPK modeling are shown in Supplementary Table S5. For calibrating model parameters, we considered time-dependent partition coefficients in kidney, liver, stomach, and intestine due to the varying exposure Cu concentration and high accumulative capacity. The fitted functions of time-dependent partition coefficient were given in Supplementary Table S6. We found that the calibrated partition coefficients in gills, muscle, bone, and carcass in chronic exposure were much higher than those of calculated data obtained from experiment.

By incorporating the calibrated physicochemical parameters, the proposed pulsed Cu-tilapia PBPK model were used to simulate the tissues-specific Cu accumulation during 10 and 28 days exposure periods. The results

demonstrated that the simulation outcomes from acute/chronic pulsed Cu-tilapia PBPK model were consistent with the experimental data on organ/tissue-specific time-dependent Cu accumulations (on average, MAPE values ranging from 20 to 50 %) (Fig. 5). Notably, Cu concentration of carcass in acute exposure and gills in chronic exposure are <20 % MAPE value, indicating a good predicting performance (Fig. 5f, l).

Mutlivariate analyses on toxicokinetics

Figure 6 shows the significant correlation between organ-specific acute and chronic Cu accumulations. A linear regression analysis revealed a significant positive correlation among acute Cu accumulation in whole body, gills ($r = 0.62$), intestine ($r = 0.92$), liver ($r = 0.75$), and kidney ($r = 0.88$), whereas there was a negative correlation ($r = -0.55$) between whole and bone. Significant correlations of acute pulsed Cu accumulation were found between blood and gonad ($r = 0.90$), gills and intestine ($r = 0.72$), gills and kidney ($r = 0.63$), muscle and carcass ($r = 0.77$), muscle and bone ($r = 0.57$), liver and kidney ($r = 0.54$), liver and intestine ($r = 0.76$), intestine and kidney ($r = 0.89$), and carcass and bone ($r = 0.89$). However, significant negative correlations occurred in bone and liver ($r = -0.57$) and in bone and intestine ($r = -0.56$). The detail specific-tissue correlations under acute exposure were shown in Supplementary Fig. S2 and Table S7.

We also found that chronic pulsed Cu accumulation in whole body had significant correlations with intestine ($r = 0.56$), stomach ($r = 0.69$), and liver ($r = 0.61$) (Fig. 6, Supplementary Fig. S3; Table S7). Moreover, chronic pulsed Cu accumulation of gonad had the significant positive correlation with intestine ($r = 0.80$) and muscle ($r = 0.61$) that were similar to acute pulsed Cu accumulation (Fig. 6, Supplementary Fig. S3; Table S7). The similar results between liver and bone with the negative correlations were also found in acute and chronic pulsed Cu exposures.

To investigate the functional similarity across organs/tissues at acute and chronic Cu exposures, we performed the Ward's minimum variance method based on the experimental dataset that was divided the clusters branch into richly perfused and poorly perfused tissues (Fig. 7). Acute exposure dendrogram indicated that groups of muscle, carcass, and bone before, during, and after pulse #2 were functionally similar, whereas gills groups during and after pulse #1 were most similar to those of muscle and carcass. On the other hand, groups of whole body are found to be most similar to those of gills before, during, and after pulse #2. However, the richly perfused tissues groups were

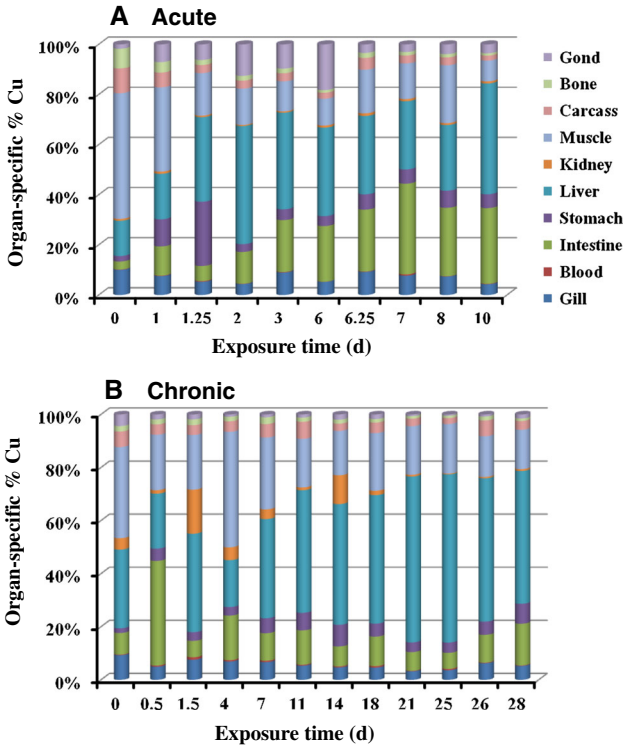

Fig. 3 Trend of organ-specific % Cu fluctuated with pulsed waterborne Cu exposure in **a** acute and **b** chronic exposure bioassays

no difference between the pulsed and non-pulsed exposure periods (Fig. 7a).

Chronic exposure dendrogram showed that accumulation similarity was not obviously different between pulsed or non-pulsed exposure periods in that most individual tissues were performed to be as one single cluster, especially in whole body and kidney (Fig. 7b). Hence, this present cluster analysis indicated that whole body, gills, bone, carcass, and blood of tilapia could be approximately classified as one group, whereas the other classified groups covered liver, kidney, intestine, and stomach. Acute exposure dendrogram showed that tissue accumulation of pulsed or non-pulsed periods to be as one single cluster (accumulation similarity). It means the accumulative level depend on dramatic external concentration changes in acute pulsed exposure, whereas the chronic pulsed Cu exposure was not related to pulse conditions (Fig. 7).

Discussion

The analytical approach we described provides an opportunity to examine and quantify metal accumulation dynamics for fish in response to environmental variability-induced non-uniform metal exposures on an organ/tissue-dependent scale and to integrate qualitative information with toxicokinetic and physiological data. Such organ/

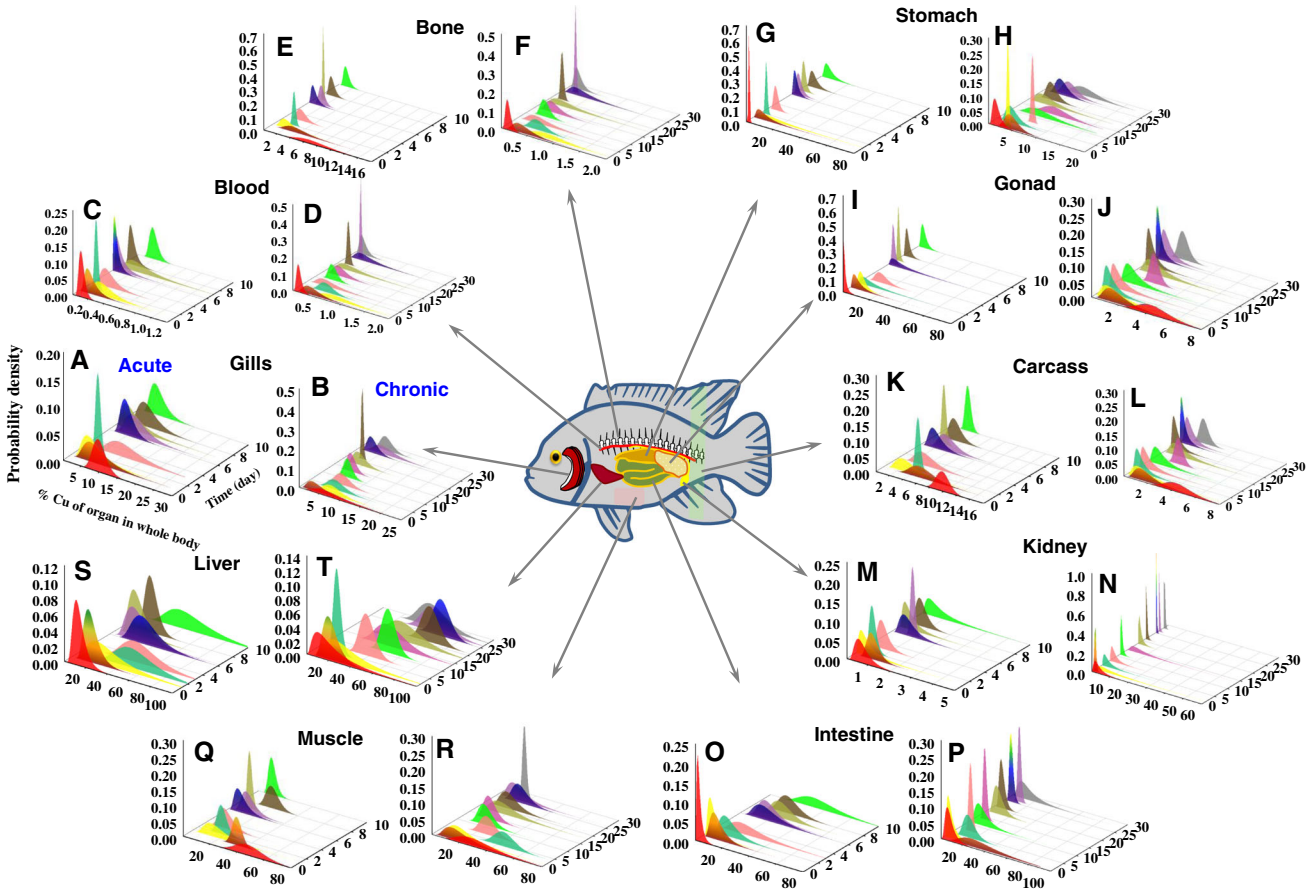

Fig. 4 Dynamics of probability distribution of % Cu of organ/tissue in whole body of tilapia exposed to acute (**a**, **c**, **e**, **g**, **i**, **k**, **m**, **o**, **q**, **s**) and chronic (**b**, **d**, **f**, **h**, **j**, **l**, **n**, **p**, **r**) pulsed waterborne Cu

tissue-dependent scale measurements of metal accumulation dynamics are a fundamental step in understanding the effects of metal toxicity on coping mechanisms of aquatic organisms. Overall, our study found that (i) stomach and kidney had the highest uptake and elimination capacities undergone the acute and chronic pulsed exposures, (ii) liver was prone to a highest BCF and was more sensitive to both acute and chronic pulse Cu exposures than the other tissues, (iii) most of the chronic organ/tissue BCFs were less than that in acute exposure, and (iv) Cu accumulation in liver, stomach, and intestine were strongly dependent on the exposure pulse characteristics such as frequency and duration and not on concentration (i.e., amplitude).

Atli and Canli (2011) indicated that kidney displayed the higher capacities on metal accumulation and metal-binding protein synthesis in *O. niloticus*. De Boeck et al. (2003), Peyghan et al. (2003), and Soedarini et al. (2012) pointed out that liver is the key site for accumulating Cu and controlling homeostatic. To date, there are no available researches that could accurately elucidate how fish tissue regulates the pulsed Cu accumulation resulting from the environmental variability. The different accumulation levels between short-term and long-term exposures may due in part to the acclimation mechanisms (Diamond et al. 2006). Gill regulation is a time-dependent acclimation characterizing by both how quickly the acclimation is activated to prevent further effects and how long the acclimation stays in place (Diamond et al. 2006). We found that BCFs increased with increasing exposure time in most of the organ/tissue in the acute pulsed Cu exposure, whereas BCFs decreased with increasing exposure time was observed in chronic exposure. The different uptake and elimination parameters may not suitable for all accumulative patterns under exposure scenarios. This study has been taken into account the accumulative level under different Cu concentration exposures for estimating the uptake and elimination rate constants. Hence, this study only considered that the estimates of uptake and elimination rate constants are dependent on pulsed period, frequency and intensity of repair capacity for a specific life stage.

In particular, liver presented highly accumulative pattern and did not decrease Cu burden even followed the low waterborne Cu in the pulsed exposure. Kotze et al. (1999) indicated that *O. mossambicus* accumulated higher Cu

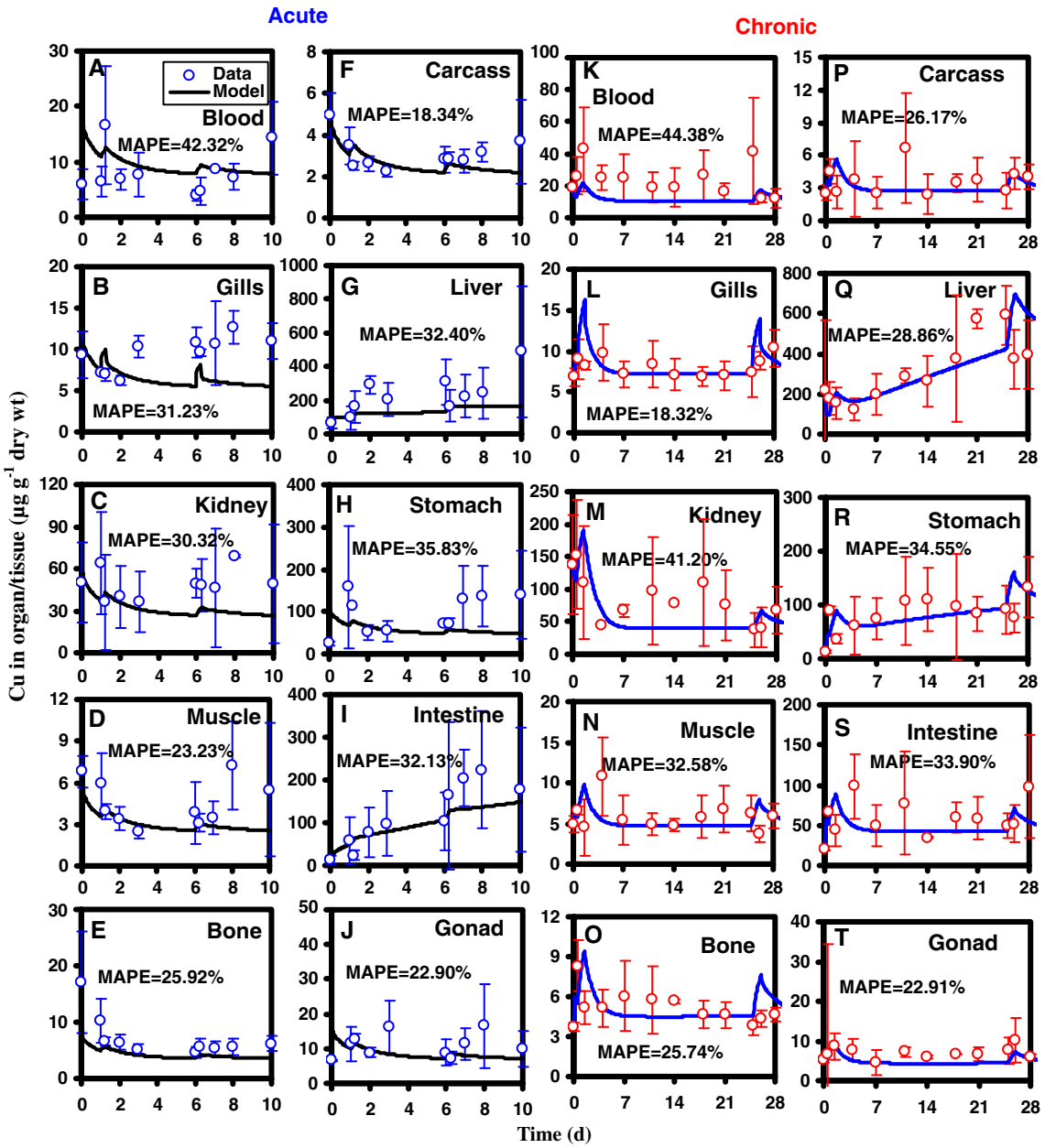

Fig. 5 Comparison of the Cu-tilapia PBPK model outcomes with measured Cu burden in blood, gills, kidney, muscle, bone, carcass, liver, stomach, intestine, and gonad of tilapia exposed to acute (**a–j**) and chronic (**k–t**) pulsed waterborne Cu

concentration in liver than other tissues. Our study showed that Cu burden in liver ranged from 66 to 600 μg g^{-1} during the acute and chronic pulsed exposures; that was in agreement with Kotze et al. (1999). Couture and Rajotte (2003) indicated that liver could promote the regulatory mechanisms when accumulated Cu burden was <50 μg g^{-1} dry wt. If liver burden exceeded that threshold, Cu accumulation will increase due to the breakdown of homeostatic control. Our study found that the homeostatic control mechanism in liver was overloaded in the present pulsed exposure situations.

We also showed that Cu accumulation distributions in liver and muscle present different temporal trends at acute and chronic pulsed exposures, even Cu accumulation concentrations were increasing in the end of exposure. However, muscle dominated high % Cu distribution in total Cu accumulation at initial exposure time and decreased % Cu distribution in the end of exposure (acute: 49 → 10 %; chronic: 33 → 15 %). On the contrary, liver substantially increased % Cu distribution in the end of exposure (acute: 12 → 45 %; chronic: 19 → 50 %). Liver presented higher Cu concentration distribution than muscle under metal

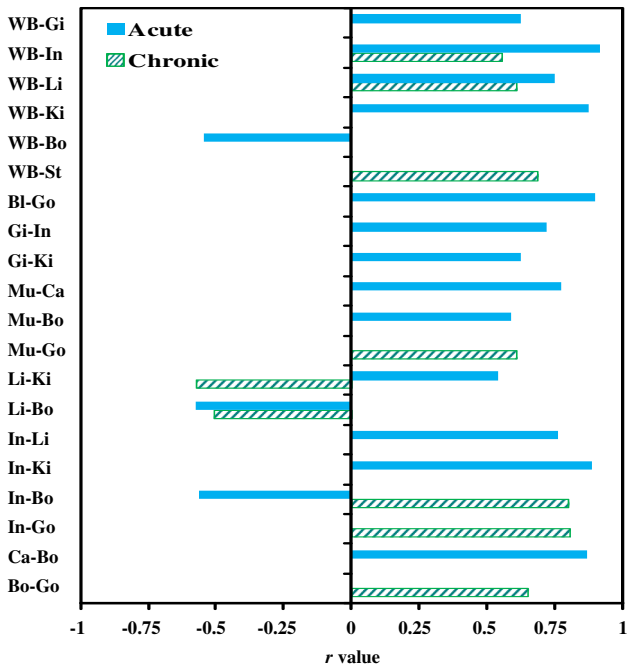

Fig. 6 Significant correlations (justified by Spearman correlation *r*) between Cu accumulations in the studied tilapia organ/tissue exposed to acute and chronic pulsed Cu. Non-significant correlation are not showed. *WB* whole body, *Gi* gill, *In* intestine, *Ki* kidney, *Bo* bone, *Go* gonad, *Mu* muscle, and *Ca* carcass

exposure since liver could rapidly uptake the newly accumulated Cu (Grosell et al. 2001).

Our present pulsed Cu-tilapia PBPK models was capable of elucidating the Cu accumulation in tissues of tilapia under different pulsed exposure scenarios. However, the pulsed Cu-tilapia PBPK model is also associated with particular restriction and disadvantages that are needed to be identified before extensively implement. The partition coefficient in the PBPK model is a key parameter in affecting concentration accumulated in organs/tissues. The partitioning of chemical between blood and target tissue can be calculated by dividing the concentration in tissue by the concentration in blood under the steady-state condition. However, this approach did not suitable for the pulsed exposure scenarios. Our study incorporated the time-dependent partition coefficient into the present Cu-tilapia PBPK model to predict Cu distributions in liver, kidney, intestine, and stomach under pulsed exposures and could adequately explain the experimental data.

The estimations of contamination distribution between blood-tissue are performed by tissue concentration under steady-state condition mostly. If the chemical is fluctuating with time, the physicochemical-physiological processes are unlikely to reach the steady-state for certain specific tissues. Weijs et al. (2010) indicated that partition coefficients of tissue/blood might change since circumstance variation, such as nutritional status. Previous studied also developed PBPK model to assess polychlorinated biphenyls and cyclosporine concentrations by using the time-dependent shift in liver and other target tissue (Lee et al. 2002; Kawai et al. 1998). Hence, it is important to incorporate the variation of partition coefficient in critical tissue that depends on the chemical exposure patterns and frequency, especially for the exposure circumstance is not stable.

This study found that low pulsed intensity and frequency did not cause the dramatic accumulation changes, whereas those of higher pulsed intensity and frequency did. Therefore, the toxicokinetic parameters or partition coefficient, etc., of acute pulsed Cu exposure would not suitable to implicate in any short-term pulsed, high pulsed intensity and frequency situation in further accumulative pattern applications. However, low pulsed frequency happened in the chronic pulsed Cu exposure, the accumulative levels present the similar states between each pulsed exposures (pulsed timing) due to long repair time.

A central goal of this paper is to provide a mechanistic and statistic description of systems-levels modeling of fish after exposure to pulses of waterborne Cu. We aim to motivate and develop effective systems-level oriented toxicokinetic model determined in a fully quantitative fashion to develop robust approximate relations that convey how environmental fluctuating contaminants impacts on fish tissue/organ responses. More generally, we hope that this paper will serve to bridge two largely distinct communities: those developing and employing new toxicological models, and those who have developed and mastered experimental techniques for understanding these accumulation, distribution, and acute/chronic exposure duration of pulsed chemical exposures for aquatic organisms. A major difficulty in studying ecotoxicological modeling from a systems perspective has been the lack of information regarding timing and sequence in which organisms are exposed to chemicals. We anticipate that one way to address this issue is to develop a mathematical framework that estimates the potential advantage of a conditioned response in a given fluctuating or pulsed environment.

In conclusion, our systems-level approach helps revealing how previously known toxicokinetics under pulsed conditions are combined to affect ecophysiological responses to environmental variability. Despite more than half of the organs/tissues of tilapia being involved in the dynamic response of accumulation to pulse Cu, our methodology could discern the key organ/tissue that regulates the pulsed Cu exposure events. Our study implicates that the overall control design for aquatic organisms in response to pulse toxicant exposure depends on quantitative system characteristics that can be achieved by controlling pulse frequency and duration. The dynamic data

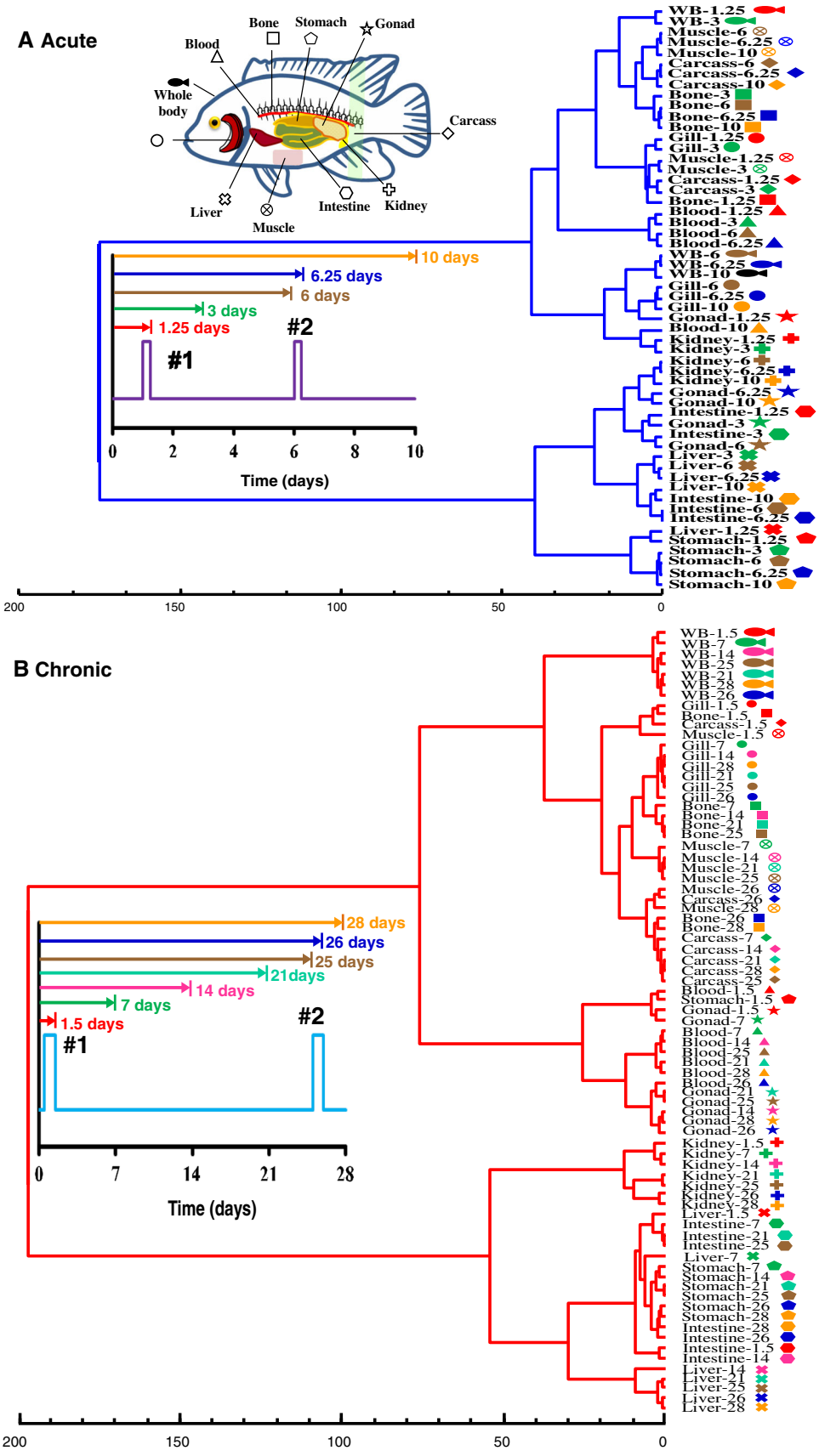

Fig. 7 Cluster analysis of tilapia organ/tissue accumulations related the combine dataset of AUC of Cu accumulation, uptake rate constant, elimination rate constant, BCF, and coefficient in **a** acute (pulsed 1: day 1–1.25, pulsed 2: day 6–6.25) and **b** chronic (pulsed 1: day 0.5–1.5, pulsed 2: day 25–26) pulsed waterborne Cu (the *symbol* and *color* represent the organ/tissue and exposure time, respectively) (Color figure online)

presented here may be used for further computational analyses such as multivariate statistics and large-scale structural or toxicokinetic models (Görlitz et al. 2011; Clements et al. 2013). We hope that our systems-level tools for mathematical analyses and modeling will facilitate future large-scale and dynamic systems biology studies in other model fish.

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Conflict of interest The authors declare that they have no conflict of interest.

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