Effect of Copper On Bioconcentration of Benzotriazole Ultraviolet Stabilizers (BUVSs) in Common Carp (*Cyprinus Carpio*)

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Abstract

Benzotriazole ultraviolet stabilizers (BUVSs) have received increasing attention due to their widespread usage, ubiquitous detection and their adverse ecological effect. However, information about the bioaccumulation potential of BUVSs and their joint exposure with heavy metals remains scarce. In this study, we investigated the bioaccumulation kinetics of 6 frequently reported BUVSs in common carp under different Cu concentration for 48 d, and their tissue-specific distribution patterns (liver, kidney, gill, and muscle tissues) were also evaluated. The bioconcentration factors (BCFs) and half-lives ($t_{1/2}$) in the tissues ranged from 5.73 (UV-PS) to 1076 (UV-327), and 2.19 (UV-PS) to 31.5 (UV-320) days, respectively. The tissue-specific concentration and BCF values followed the order of liver > kidney > gill > muscle with or without Cu exposure. An increase in BCF with rising Cu concentration was observed, which is caused by the decreased depuration rate (k_2) in more than half of treatment groups. These results indicated that BUVSs accumulated in fish and provides important insight into the risk assessment of this group of chemicals.

1. Introduction

Ultraviolet (UV) radiation can cause aging of organic materials as well as human skin, posing a threat to both environment and human body (Correa et al., 2021; Zeng et al., 2018). As a result, UV stabilizers have been widely applied into industrial products and personal care products (PCPs). Among all UV stabilizers, benzotriazole ultraviolet stabilizers (BUVSs) have the largest output and most variety (Li and Li, 2007). BUVSs can absorb full spectrum of UV light from 280 to 400 nm (UV-A and UV-B), and they are widely used as additive in building materials, paint, plastics or sunscreens, creams and shampoos (Kim et al., 2011a; Nakata et al., 2009). Some BUVSs (like UV-234, UV-328, etc.) have been listed as High Production Volume Chemicals (HPVC) by the Organization for Economic Co-operation and Development (OECD) (OECD, 2017). BUVSs can be released into different environmental compartments, predominantly into aquatic system through various pathways, mainly via direct recreational activities such as bathing and swimming or indirect landfill leachate and sewage treatment plant effluents (Apel et al., 2018; Carpinteiro et al., 2010; Lu et al., 2017; Parajulee et al., 2018). And they can also be discharged into soils as a result of solid waste or emitted into air due to abrasion and volatilization (Carpinteiro et al., 2010; Xiong, 2017). Consequently, BUVSs have been detected in environmental matrices such as surface water (Parajulee et al., 2018), wastewater (Carpinteiro et al., 2010), sewage sludge (Lu et al., 2017), sediment (Nakata et al., 2009), soil (Lai et al., 2014), and indoor dust (Kim et al., 2012). Relatively few studies reported BUVSs in aquatic biota, mainly in marine invertebrates, fishes, coastal birds, and marine mammals (Hemalatha et al., 2020; Kim et al., 2012; Nakata et al., 2009), demonstrating their bioaccumulation potential. What's more, it's been reported that BUVSs are found in human urine and breast milk in recent years (Kim et al., 2019; Wang et al., 2013). Overall, these compounds are ubiquitous in the natural environment, human settlements and living organisms.

Considering the widespread presence of BUVSs, their adverse effects on organisms have been investigated. Hirata-Koizumi et al. (2009) found a gender-related hepatic peroxisome proliferative activity of HDBB in rats. And some BUVSs are proved to have partial estrogenic activity or to disturb thyroid hormone pathway, development, and locomotory activity of early-stage zebrafish (Feng et al., 2020; Liang et al., 2017). An activation of aryl hydrocarbon receptor pathway in zebrafish eleuthero-embryos was observed after their being exposed to BUVSs. Besides, these compounds are also reported to cause oxidative stress damages in *Daphnia magna* and zebrafish (Giraudo et al., 2017; Hemalatha et al., 2020). More recently, Li et al. (2019) and Li et al. (2020) revealed their inflammatory effects in fish, which was potentially caused through the AHR-IL17/IL22 pathways.

BUVSs have been considered to be persistent in environment (Nakata et al., 2010) and their lipophilicity/hydrophilicity (log *K*ow:4.31-8.28) property is the main factor governing their accumulation potential in aquatic organisms (Hemalatha et al., 2020; Xing et al., 2018). For instance, UV-327 showed a significant bioaccumulation property in marine mammals from the western North Pacific Ocean with a bioconcentration factor (BCF) value as high as that of persistent organochlorine pesticide, hexachlorocyclohexane (37 000) (Hemalatha et al., 2020). In addition, high bioaccumulation factor (BAF) of BUVSs were also observed in fresh water aquatic organisms from the Pearl River basin in China, and some of the BUVSs congeners showed trophic magnification behavior with trophic magnification factor (TMF) > 1 (Xiong, 2017). More recently, Zhang et al. (2021) investigated the accumulation and biotransformation of 6 BUVSs in zebrafish under controlled laboratory conditions and reported the BCF values at the range of 1.04-10400 in different fish tissues.

On the other hand, heavy metals such as Cd, Cr, Pb, Cu, Zn, etc., have been widely detected in environmental media including sediments, water, and soils (Li et al., 2015; Peng et al., 2022; Zhang et al., 2018). Among these heavy metals, copper draws a great concern. The average concentration of copper in China's major river basins ranged from 11.72 to 527.77 $\mu g \cdot L^{-1}$ in 2006-2017 (He et al., 2019). And the co-contamination by heavy metals and organic contaminants is attracting increasing attention, as they both can be persistent and bioaccumulative in environment (Zhao et al., 2018b). In previous studies, Zhao et al. (2018a) found that copper could increase the bioaccumulation of Fluoroquinolones (FQs) in zebrafish tissues. Recently, Zhang et al. (2021) reported a decline in the concentration of PFAAs (C_2-C_8) in wheat root by 6%-73% due to excessive Cu exposure, while the translocation of long-chain per□uorooctanoic acid (PFOA) and per□uorooctane sulfonic acid (PFOS) was promoted and positively associated with the Cu exposure levels. However, studies on the joint effect of copper and BUVSs are still lacking.

In present studies, combined effects of benzotriazole and copper on organisms have been evaluated, considering benzotriazole is widely used as corrosion inhibitor for copper and its alloy (Grillo et al., 2014; Xing et al., 2017; Xing et al., 2018). As a subgroup of benzotriazole with a phenolic group attaching to the benzotriazole structure, BUVSs are also widely used as additives in automobile components and some sports equipment etc (Nakata et al., 2009). Therefore, in this study, we investigated the bioconcentration and distribution pattern of BUVSs in different tissues (liver, kidney, muscle, and gill) of common carp (*Cyprinus carpio*) and evaluated the effect of copper on their bioaccumulation, which provided a first glimpse into the co-contamination by heavy metals and BUVSs.

2. Materials And Methods

2.1. Chemicals

Standards of UV-PS, UV-320, UV-326, UV-327, UV-328, UV-329, and surrogate standards Allyl-bzt and phenanthrene-d_{10} were purchased from Sigma-Aldrich (St. Louis, MO, USA). Purities of all the standards were not less than 98%. The physicochemical characteristics of BUVSs are shown in Table S1,Table S2. Analytical grade chemicals, anhydrous sodium sulfate (Na_2SO_4) (60 mesh) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and neutral silica gel (100-200 mesh) was purchased from Thermo Fisher Scientific Co., Ltd. Solvents, including dichloromethane, isooctane, and n-hexane, were of HPLC grade (Sigma-Aldrich, St. Louis, MO, USA). Ultrapure water was obtained from an ultrapure water system produced by Shanghai Hitech Instruments Co., Ltd.

Individual stock solutions of each BUVSs (1g·L^{-1}) were made in isooctane. Further dilutions and mixtures of target species were prepared in n-hexane. All the working solutions were stored in brown glass bottles at −20°C.

2.2. Uptake and depuration experiments

The experiments were conducted in a semi-static aquarium system with constant aeration. Common carp (5.8 ± 0.6 cm, 3.3 ± 0.7 g) were obtained from a local aquarium in Dalian, Liaoning Province. Fishes were acclimated for two weeks with dechlorinated tap water (dissolved oxygen 7.9 mg·L^{-1} ± 0.5, pH 7.3-8.0, and at 23 ± 1°C) in advance.

Common carp were exposed to a mixture of six frequently detected BUVSs under constant concentration (10 μg·L^{-1} for each) in glass tanks containing 100 L of water. According to OECD 305, the chemical concentration in water for bioconcentration tests should not exceed 1% of its 96 h LC_{50}. Considering a relatively high LC_{50} of six BUVSs (>10 mg·L^{-1}) (Kim et al., 2011a), the exposure concentration in this study is a bit higher than reported environmental concentrations (2.3-307.7 ng·L^{-1} (Kameda et al., 2011)), while far less than the LC_{50}. Three exposure groups were set as Cu-blank, Cu-low (2.56 μg·L^{-1}) and Cu-high (25.6 μg·L^{-1}), respectively. And for each exposure group, there were two parallel groups. Besides, there was a non-spiked tank served as blank group.

Three fish were sampled randomly from each tank on d 2, 4, 8, 12, 16, 20, 24, and 28 during exposure period and on d 30, 32, 36, 40, 44 and 48 during depuration period. Half of the water in tanks were renewed over a period of 24 h to keep the test solution fresh. The length and weight of each fish were measured after being sampled. Fish were anesthetized on ice and then dissected, their four tissues including liver, kidney, gills, and muscle were carefully removed, weighed and stored at −20°C for subsequent analysis.

2.3. Sample extraction and analysis

Sample pretreatment was performed using a procedure described by Kim et al. (2011b) and Carpinteiro et al. (2010) with some modifications. After being homogenized in mortar with the addition of 200 ng internal standard (Allyl-bzt) and anhydrous sodium sulfate, the fish tissue was transferred into 50 mL polypropylene centrifuge tubes. And the samples were extracted with a mixture of dichloromethane and n-hexane in a volume ratio of 8:1. For a better interaction between solvent and sample, the tube was vortexed for 1 min then deposed into ultrasonic bath for 30 min. Hereafter, the tube was centrifuged at 12,000 rpm for 10 min. The extraction step was repeated twice and the supernatant was evaporated to dryness under a gentle stream of nitrogen with heating. The residue was reconstituted in 2 mL n-hexane for a further clean-up by glass column loaded with anhydrous sodium sulfate and silica gel. Dichloromethane was used for eluting and then evaporated to dryness and reconstituted in 1 mL n-hexane then filtered through a 0.22 μm membrane filter for GC-MS analysis. The details of GC-MS analysis and quantitation are listed in the Supporting Information (Text S1 and Table S2). Extractions of BUVSs in water samples followed the steps in the Supporting Information (Text S2).

2.4. Quality assurance and quality control (QA/QC)

All glasswares and mortars being used were calcined in muffle furnace at 400°C for 4 h in advance. Procedural blanks going through the same treatment as samples were determined with each batch to monitor potential contamination during analysis. The method detection limit (MDLs) and method quantification limit (MQLs) were calculated as three and ten times the standard deviation (SD) of the mean procedural blanks (n = 6), and ranging from 0.05 to 0.81 ng·g^{-1} wet weight (ww) and 0.15 to 2.70 ng·g^{-1} ww, respectively. Fish tissues spiked with standards of 3 levels were detected to processed the reproducibility and accuracy of method (n = 3). The recoveries ranged from 85.6–111.2% with relative standard deviations (RSD) ranging from 6.9–23.5% (Table S4).

2.5. Data analysis

Bioconcentration kinetic parameters were estimated with a mass balance model (Mackay and Fraser, 2000), where uptake and depuration process can be described by eq. (1):

$$\frac{dC_B}{dt} = k_1 C_W - k_2 C_B \quad (1)$$

Where C_B (ng·g^{-1}, ww) and C_W (ng·L^{-1}) are concentrations of chemicals in fish tissue and aquatic environment, respectively. k_1 (L·(kg·d)$^{-1}$) and k_2 (d^{-1}) are the rate constants for uptake and depuration process, respectively. t is the exposure time (d). In this study, the elimination of BUVSs caused by metabolism and growth were ignored. Then k_1 can be calculated by fitting a nonlinear regression to the eq. (2):

C_B=[(k_1/k_2)C_W](1-e$^{-k_2t}$) (2)

And k_2 can be obtained by eq. (3):

C_B=Ae$^{-k_2t}$ (3)

In this case, the BAF^{∞}_{fish} would correspond to eq. (4):

$BCF=\frac{k_1}{k_2}$ (4)

And the half-lives ($t_{1/2}$) of BUVSs in fish samples can be calculated according to the eq. (5):

$t_{1/2} = \frac{\ln 2}{k_2}$ (5)

Statistical analyses were performed using Origin 2017 (Origin Lab Corporation) and SPSS 22 (IBM Co, Armonk, NY, USA). Paired-samples T-test was applied to examine the significance difference among treatment groups. A p-value of < 0.05 was accepted as statistically significant.

3. Results And Discussions

3.1. Fish mortality and morphological indices

During the experimental period, fish mortality was less than 3% and no abnormal behavior was observed in both control and treatment groups. Hepatosomatic index (HSI) is one of the most frequently used morphological indices to evaluate the effect of test solution on individual fish (Li et al., 2011), thus HSI of fish was determined in this study, calculated by the ratio of liver weight to whole fish weight. No significant difference was shown either between treatment groups and control group or among treatment groups (Paired Student's t-test; P > 0.05). Details of fish mortality and HSI values are provided in Table S5 and S6.

3.2. Uptake and depuration of BUVSs

During the exposure periods, the concentrations of 6 BUVSs in water were maintained relatively stable (10 μg·L^{-1}) (Table S7). The bioconcentration profiles of 6 BUVSs in common carp under different Cu concentrations are presented in Fig.1. For most of the groups, BUVSs showed similar accumulation patterns, except for UV-PS, UV-326, and UV-329 in muscle, where no increasing tendency was observed. In the uptake phase, the concentrations of BUVSs kept increasing, rapid uptake of BUVSs was observed in all treatment groups. As for the depuration phase, the concentrations of BUVSs showed a sharp drop in the first 2-4 days then decreased slowly. What's more, most BUVSs remained at a comparatively high level in some fish tissues at the end of the depuration phase, suggesting their latent high persistence in fish.

When it comes to the effect of copper, we found significant differences between BUVSs concentrations in fish tissues under different Cu exposure (19 groups out of 21, P < 0.05, Fig. S1). For more than half of the treatment groups (12 groups out of 19), the concentrations of BUVSs decreased as Cu concentration increasing (Fig. 2), suggesting an inhibitive effect of Cu on the accumulation of BUVSs. However, in the rest of the treatment groups (mostly in kidney 3/7 and muscle 2/7), the concentrations of BUVSs under low Cu concentration were apparently higher than those in another two groups (Fig. 2), which means low Cu concentration may also stimulate the accumulation of BUVSs in fish.

It has been reported that benzotriazole can significantly increase the bioaccumulation of Cd in zebrafish as well as Cu in earthworms (Duan et al., 2017; Xing et al., 2018). While the combined effects of binary benzotriazole and Cu mixture were found to be antagonistic on *Eisenia fetida* earthworm avoidance behavior and its survival (Xing et al., 2017). Besides, their combined exposure also reduces the oxidative stress in earthworms induced by single compound (Zhang et al., 2020). Stable complexes are found between benzotriazole and Cu as for the nitrogen sp^2 lone pairs of benzotriazole, thus changing the bioavailability of this compound (Grillo et al., 2014). Having a similar structure with benzotriazole, BUVSs may show alike interaction with metals, thus causing different combined effects, as is shown in our results. Besides, the reduction in bioaccumulation of organic compounds under Cu co-exposure was reported in other studies. Feng et al. (2015) found an apparent decrease in the bioaccumulations of PFOS in fish tissues under Cu exposure. And the authors deduced a synergistic effects of PFOS, PFOA, and Cu on sh liver. Deng et al. (2018) observed that the contents of polycyclic aromatic hydrocarbons (PAHs) in the shoots and roots of pakchoi were signficantly lower under PAHs + Cu treatment compared with PAHs only, the reason of which might be due to their complicated interactions, and they were found to impede each other biological adsorption or engage in the noncovalent molecular interaction (cation − π interaction).

3.3. Bioconcentration factor of BUVSs

The uptake and elimination kinetics of 6 BUVSs in liver, kidney, gill, and muscle of fish for all treatment groups were investigated respectively. The uptake, elimination rate constants (k_1, k_2) were acquired through the mass balance model (Mackay and Fraser, 2000), results are shown in Table 1. Without Cu exposure, the k_1 values of 6 BUVSs ranged from 1.24 to 48.43 in liver, 1.81 - 36.13 in kidney, 0.43-17.67 in gill, and 6.40-7.80 in muscle, while the k_2 values were in the range of 0.05-0.31, 0.05-0.32, 0.04-0.31 and 0.05-0.09 in the above-mentioned tissues, respectively.

Subsequently, half-lives ($t_{1/2}$) and BCF values were calculated based on k_1 and k_2 (Table 1). The BCF values of 6 BUVSs increased in the order: UV-PS < UV-329 < UV-326 < UV-328 < UV-320 < UV-327. The BCF values in liver and kidney tend to be apparently higher than those in gill and muscle. These results were also observed in the study of BUVSs bioconcentration in zebrafish (Zhang et al., 2021), though the BCF values in our study were lower. According to Zhang et al. (2021), the higher BCF values in zebrafish can be explained by their smaller body size with larger ratios between skin surface area and body weight, enabling more possible absorption for chemicals. Among the 6 BUVSs, UV-327 had the highest BCF value of 1076 (in liver), which is comparable to that reported on European Chemicals Agency (ECHA, 2013) (900 with the test concentration of 10 $\mu g \cdot L^{-1}$ in common carp). While the lowest BCF (5.73, in kidney) was calculated for UV-PS. Besides, the BCF values of UV-326 in our study (61-222) are similar to those reported by Nakata et al. (2009) (196-802 at the exposure concentrations to 0.05 $mg \cdot L^{-1}$ in carp).

Table 1

Kinetic parameters of 6 BUVSs in common carp at concentration of 10 μg·L^{-1} under different Cu concentrations.

		UV-320 Cu-blank	UV-320 Cu-low	UV-320 Cu-high	UV-327 Cu-blank	UV-327 Cu-low	UV-327 Cu-high	UV-328 Cu-blank	UV-328 Cu-low	UV-328 Cu-high
Liver	k_1[a]	29.02±8.72	69.01±4.08	44.32±4.16	48.4±11.02	53.39±8.87	131.1±23.59	29.42±4.86	23.83±3.90	34.25±7.68
	k_2[b]	0.07±0.03	0.11±0.04	0.02±0.00	0.05±0.02	0.06±0.00	0.03±0.01	0.10±0.04	0.07±0.03	0.10±0.05
	BCF[c]	403.04	657.22	2014.36	1076.22	970.75	4370.37	294.15	331.01	356.72
	$t_{1/2}$[d]	9.63	6.6	31.5	15.4	12.6	23.1	6.93	9.63	7.22
Kidney	k_1[a]	32.77±3.18	32.90±3.13	26.95±6.79	36.13±6.65	38.41±4.62	43.53±4.62	19.10±2.41	13.85±0.98	17.08±2.96
	k_2[b]	0.05±0.02	0.07±0.03	0.06±0.02	0.07±0.03	0.06±0.02	0.05±0.02	0.08±0.03	0.07±0.03	0.04±0.01
	BCF[c]	630.21	463.37	427.81	494.92	685.91	870.56	232.95	203.65	461.57
	$t_{1/2}$[d]	13.33	9.76	11	9.49	12.38	13.86	8.45	10.19	18.73
Gill	k_1[a]	17.67±2.10	18.05±2.05	12.81±1.72	10.42±0.91	13.25±1.35	12.55±2.17	5.40±1.84	5.69±0.77	4.62±0.81
	k_2[b]	0.10±0.04	0.040±0.01	0.04±0.02	0.04±0.02	0.04±0.01	0.04±0.01	0.31±0.12	0.09±0.04	0.06±0.02
	BCF[c]	171.6	384.1	297.8	242.4	323.2	348.7	17.2	61.87	82.54
	$t_{1/2}$[d]	6.73	14.74	16.12	16.12	16.9	19.25	2.21	7.53	12.38
Muscle	k_1[a]	7.30±1.98	8.42±0.67	6.92±0.99	6.40±1.11	5.44±0.92	6.55±1.14	7.78±4.04	3.61±0.41	2.91±0.63
	k_2[b]	0.05±0.02	0.06±0.03	0.03±0.01	0.06±0.02	0.09±0.03	0.02±0.01	0.09±0.03	0.14±0.05	0.12±0.04
	BCF[c]	152.1	153.1	209.6	112.2	64.05	297.7	87.45	25.4	25.28
	$t_{1/2}$[d]	8.88	12.38	13.08	12.16	4.95	19.8	4.88	2.86	3.98
		UV-329 Cu-blank	**UV-329 Cu-low**	**UV-329 Cu-high**	**UV-PS Cu-blank**	**UV-PS Cu-low**	**UV-PS Cu-high**	**UV-326 Cu-blank**	**UV-326 Cu-low**	**UV-326 Cu-high**
Liver	k_1[a]	1.24±0.24	2.44±0.39	2.11±0.35	3.41±0.76	1.87±0.37	1.23±0.24	20.93±4.85	12.73±3.16	15.00±3.13
	k_2[b]	0.14±0.04	0.05±0.02	0.10±0.04	0.31±0.04	0.07±0.03	0.05±0.02	0.09±0.04	0.11±0.04	0.05±0.01
	BCF[c]	9.2	46.96	21.48	11.1	25.23	27.27	222.65	121.25	319.13
	$t_{1/2}$[d]	5.13	13.33	7.07	2.26	9.36	15.4	7.37	6.6	14.75
Kidney	k_1[a]	1.95±0.24	1.39±0.21	2.75±0.49	1.81±0.42	0.83±0.19	1.68±0.28	18.86±2.37	10.30±0.63	17.84±3.69
	k_2[b]	0.06±0.02	0.04±0.01	0.05±0.02	0.32±0.10	0.08±0.03	0.05±0.02	0.12±0.03	0.09±0.03	0.07±0.03
	BCF[c]	35.44	39.69	54.92	5.73	11.77	35	162.56	113.23	247.79
	$t_{1/2}$[d]	12.6	19.8	13.86	2.19	9.24	14.44	5.97	7.62	9.63
Gill	k_1[a]	1.50±0.18	1.33±0.08	0.67±0.14	0.43±0.09	0.45±0.03	1.05±0.04	4.35±0.63	3.09±0.55	3.70±0.42
	k_2[b]	0.11±0.04	0.12±0.03	0.14±0.03	0.05±0.01	0.05±0.01	0.06±0.02	0.13±0.05	0.07±0.02	0.10±0.03
	BCF[c]	13.65	11.56	4.65	8.37	8.73	18.71	61.21	43.52	38.89
	$t_{1/2}$[d]	6.3	6.03	4.85	13.59	13.33	12.38	9.76	9.76	7.29

[a] L·(kg·d)$^{-1}$; [b] d^{-1}; [c]L·kg^{-1}; [d]d

After exposure to different Cu concentrations, various changes were observed in different compounds from different tissues, causing a wider range of k_1 and k_2 values (1.23-131.1 in liver, 0.88-43.53 in kidney, 0.45-18.05 in gill and 2.91-8.42 in muscle for k_1, 0.02-0.14, 0.04-0.09, 0.04-0.14 and 0.02-0.14 for k_2).

The existence of Cu can significantly affect the BCF values (Fig. 3). In general, high-dose Cu exposure led to an increase of BCF values, mainly because of the apparent decrease of k_2. For the same reason, $t_{1/2}$ were also found to be longer in those groups, the longest $t_{1/2}$ (31.5 d) was recorded for UV-320 in liver under high-dose Cu exposure. In half of the treatment groups, the BCF values increased as Cu concentration increased. However, low-dose Cu might also cause a decrease of BCF values in some of the groups, which mostly occurred in liver and kidney. Besides, an opposite trend was found in a few groups, the increase of Cu concentration decreased the BCF values. Similarly, in a controlled experimental study of FQs, Zhao et al. (2018a) reported significant increase of BCF values of enrofloxacin in liver and gill tissues with decreasing Cu exposure concentration, but the opposite trend was observed for enrofloxacin and ofloxacin in muscle of zebrafish. In another study, the presence of Cu was found to decrease the uptake of PFOA by earthworms, thus causing lower biota-to-soil accumulation factors (BSAF) (Zhao et al., 2018b). It was speculated that the decrease was a result of enhanced sorption of PFOA to soil in the presence of divalent cations (Cu^{2+}), which reduced its concentrations in the pore water and desorption from ingested soil particles in earthworm gut. However, this inference cannot be applied into fish, as we conducted our experiment only in aquatic environment. Overall, further study is required to investigate the specific mechanisms of Cu and organic pollutants co-exposure in fish.

3.4. Tissue distribution of BUVSs in common carp

For a better understanding of the bioaccumulation of BUVSs in common carp, tissue distribution was investigated by calculating concentration percentages of each compound in liver, kidney, gill, and muscle at the end of the uptake phase, as shown in Fig. 4.

In general, BUVSs concentrations in fish tissues followed a trend of the order of liver > kidney > gill > muscle. Among 6 BUVSs, the largest relative composition belonged to UV-327 in liver, reaching more than 50 percent. While the combined proportion in gill and muscle was around 20 percent for most BUVSs. Similarly, Peng et al. (2020) observed that the concentration of BUVSs in fish liver is apparently higher than those in other tissues like muscle, bladder and fat. Other compounds, like organophosphate flame retardants (OPFRs), poly brominated diphenyl ethers (PBDEs), and FQs, are also reported previously to be more likely to accumulate in metabolically active fish tissues (liver or kidney) instead of in metabolically inactive tissues (muscle) (Bekele et al., 2021; Chen et al., 2019; Kim et al., 2015).

In the present study, Cu exposure did not significantly change the distribution patterns of BUVSs in fish tissue ($P > 0.05$), the order of BUVSs concentrations in each tissue remains same as that in Cu-blank groups, which is in accordance with the conclusion from the study of FQs in zebrafish (Zhao et al., 2018a) and PFOS and PFOA in *Carassius auratus* (Feng et al., 2015). However, as the concentration of Cu increased, the relative proportion of UV-PS increased while that of UV-320 and UV-327 decreased in liver. Meanwhile, their percent in kidney showed opposite trends. For UV-329, its percent increased in liver and decreased in gill. As for the proportion of BUVSs in muscle, slight variation was observed after Cu exposure.

4. Conclusion

In brief, our study indicated that the bioaccumulation of BUVSs differed among fish tissues. Their tissue-specific BCF and concentration generally followed the order of liver > kidney > gill > muscle. Besides, effect of copper on the bioaccumulation of BUVSs was investigated in this study for the first time, and the effect varied from the compounds and fish tissues. Cu exposure can apparently affect the uptake and eliminate rates of BUVSs. And an increase of BCF value caused by increasing Cu concentration was observed in half of the treatment groups (11 groups out of 21). However, Cu will not significantly alter the tissue distribution pattern of 6 BUVSs. Overall, these results provide basic data for the risk assessment of BUVSs and their co-presence with copper. Nonetheless, the mechanism of the interaction between BUVSs and heavy metals remains unclear, which requires further elucidation.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

DZ: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft

TGB: Validation, Writing – review & editing

HZ: Conceptualization, Funding acquisition, Project administration, Resources, Supervision

All authors read and approved the final manuscript.

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Figures

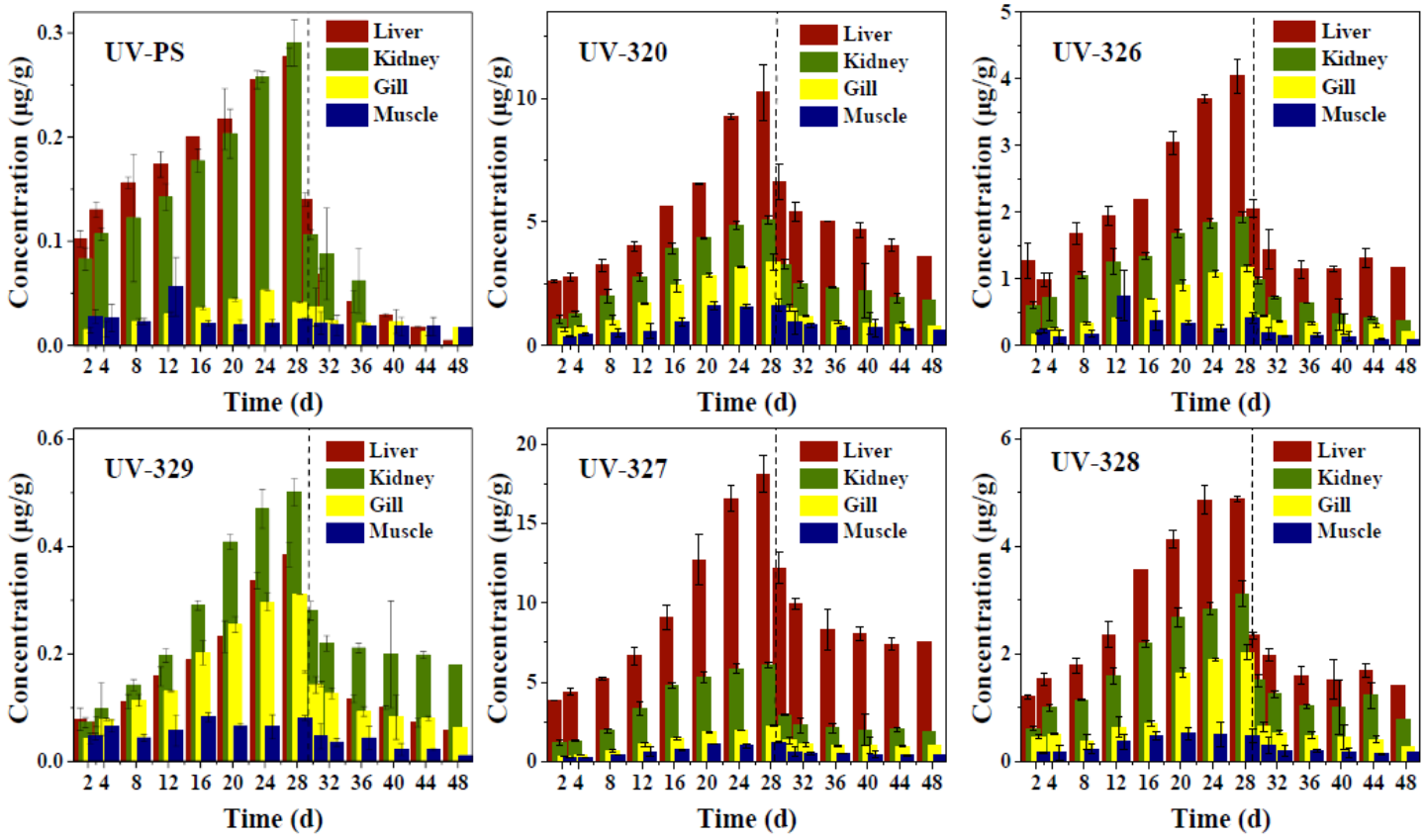

Figure 1

Uptake and depuration of target BUVSs in common carp liver, kidney, gill, and muscle exposed to BUVSs at 10 μg·L-1. The dashed line represents the day before depuration period.

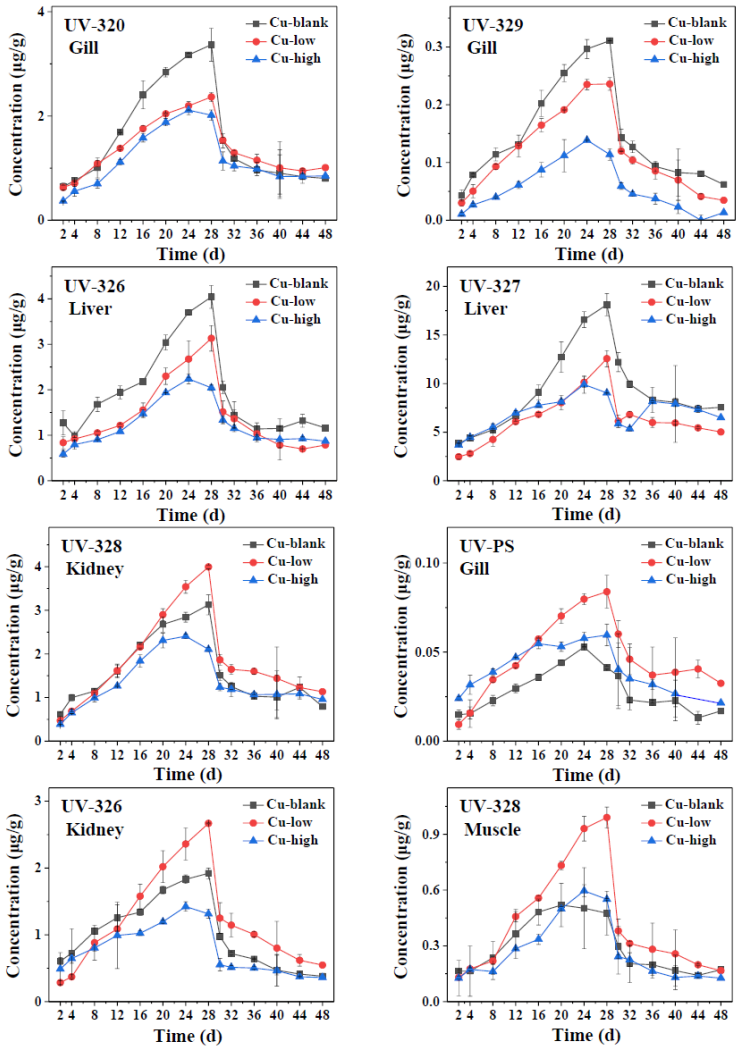

Figure 2

Uptake and depuration curves of BUVSs in tissues of common carp under different Cu concentrations.

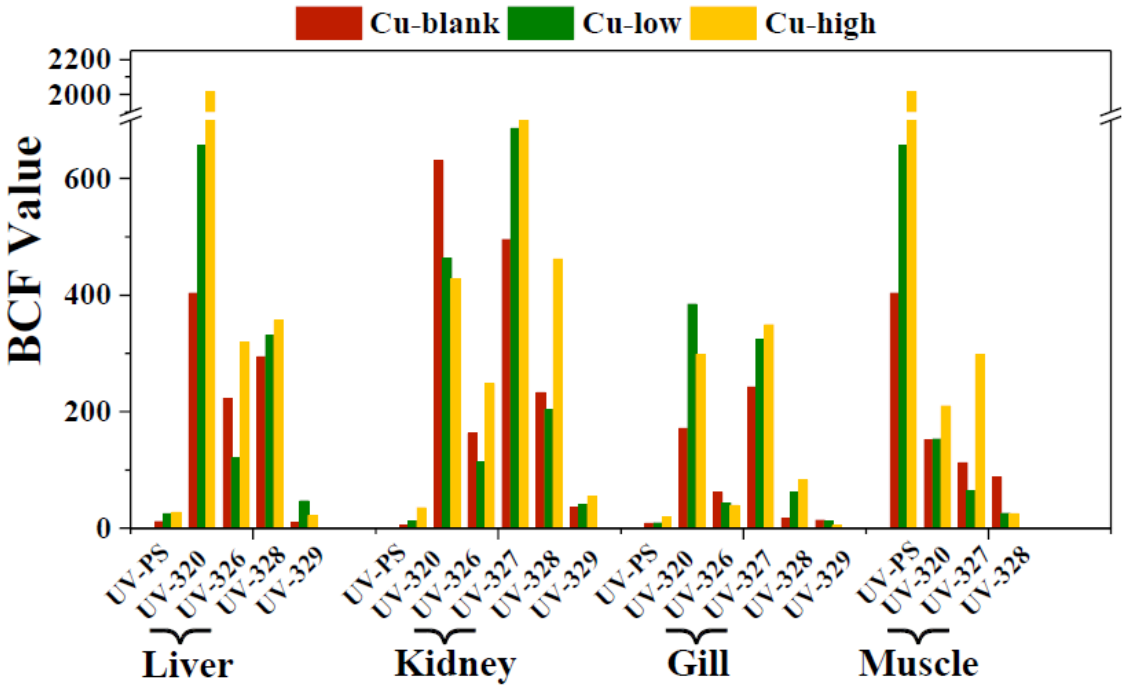

Figure 3

BCF values of BUVSs under different Cu concentration treatments.

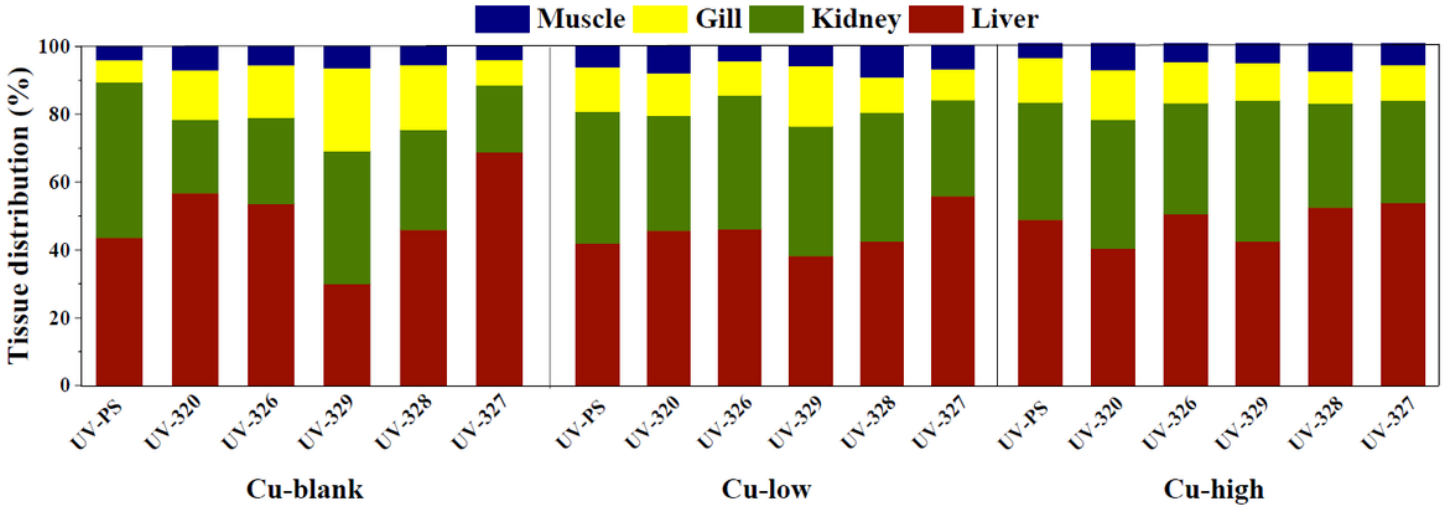

Figure 4

Tissue distribution of 6 BUVSs in common carp after 28 days exposure.

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