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# The dynamic changes of arsenic biotransformation and bioaccumulation in muscle of freshwater food fish crucian carp during chronic dietborne exposure

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ABSTRACT

Dietary uptake is the major way that inorganic arsenic (iAs) enters into benthic fish; however, the metabolic process of dietborne iAs in fish muscle following chronic exposure remains unclear. This was a 40-day study on chronic dietborne iAs [arsenite (AsIII) and arsenate (AsV)] exposure in the benthic freshwater food fish, the crucian carp (*Carassius auratus*), which determined the temporal profiles of iAs metabolism and toxicokinetics during exposure. We found that an adaptive response occurred in the fish body after iAs dietary exposure, which was associated with decreased As accumulation and increased As transformation into a non-toxic As form (arsenobetaine). The bioavailability of dietary AsIII was lower than that of AsV, probably because AsIII has a lower ability to pass through fish tissues. Dietary AsV exhibited a high potential for transformation into AsIII species, which then accumulated in fish muscle. The largely produced AsIII considered more toxic at the earlier stage of AsV exposure should attract sufficient attention to human exposure assessment. Therefore, the pristine As species and exposure duration had significant effects on As bioaccumulation and biotransformation in fish. The behavior determined for dietborne arsenic in food fish is crucial for not only arsenic ecotoxicology but also food safety.



## Introduction

Arsenic (As) is a highly toxic metalloid and well-known freshwater contaminant that poses a health risk worldwide (Du et al., 2015). The source of As in the aquatic environment is mainly human activity. For example, surficial sed-

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iment of Red Lake, Ontario, contained levels of As exceeding 5000 μg/g dry weight (dw) as a result of long-term mining and smelting of metals (Pedlar and Klaverkamp, 2002). Up to 1000 μg/g dw, As has been found in the sediments of Pearl River, China, which was caused by decades of urban and industrial activities (Jian et al., 2010). The As in sediment could be passively ingested by benthic fishes living in polluted habitats. For example, the benthic fish, *Callionymus richardsonii*, collected from Daya Bay, China, which contains 3.3 μg As/g in the sediment, exhibited up to 28.6 μg As/g in its muscle (Zhang et al., 2018). It was reported that As concentration in benthic fishes was positively correlated with As in the sediment rather than the soluble As in the water (Hong et al., 2018; Zhang et al., 2018). Ingestion of As-contaminated sediments is a crucial route of As uptake by benthic-feeding fishes. Previous studies have examined As metabolism in fishes; however, they mostly focused on soluble As in the water. Information regarding the metabolism of dietary inorganic As (iAs) in freshwater benthic fishes is very limited. Considering that the iAs species, arsenite (AsIII) and arsenate (AsV), are the main species in aquatic sediment, research on the bioaccumulation and biotransformation of dietborne iAs in fishes is of great importance (Cullen and Reimer, 2010).

It has been observed that chronic As waterborne exposure could cause As adaptation, including decreased As bioaccumulation and toxicity in fishes, *e.g.*, medaka (*Oryzias mekongensis*) (Chen et al., 2018), killifish (*Fundulus heteroclitus*) (Miller et al., 2007), and spotted murrel (*Channa punctatus*) (Allen and Rana, 2004b; Allen et al., 2004). Two possible mechanisms related to As adaptation in organisms have been proposed. First, body As levels could be balanced or reduced by regulating the elimination and uptake mechanisms. In mammal cells and fishes, the decrease of As levels was found to be correlated with elevated As elimination rates and reduced As uptake rates (Liu et al., 2001; Romach, 2000). Moreover, reduced expression of As-uptake or increased expression of As-efflux transporter proteins were related to decreased As accumulation (Lee et al., 2006; Roggenbeck et al., 2016). Second, biotransformation may also contribute to As adaptation through detoxification of iAs. The processes of iAs detoxification in organisms could include the reduction of AsV to AsIII, the oxidative methylation of AsIII to methylated metabolites (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)), and further transformation to the non-toxic arsenocholine (AsC) and arsenobetaine (AsB) as well as binding of AsIII with sulfhydryl or excretion of As out of the body (Ajees et al., 2012; Sun et al., 2016). Methylation of iAs has long been indicated as a detoxification process because MMA and DMA are less toxic than iAs in acute lethality assays (Guo et al., 2016; Stýblo et al., 2002). However, recent studies have reported trivalent unstable intermediates in the methylation process, MMAIII and DMAIII, which exhibit stronger cytotoxicity, genotoxicity, and enzyme inhibition than does iAs (Moe et al., 2016). Over the decades, the As biotransformation in fishes has been studied mostly through waterborne As exposure, and information on biotransformation of dietborne iAs is very limited.

In the present work, we initiated a feeding study for 40 days. The goal was to investigate the dynamic change in iAs metabolism in a freshwater benthic food fish, the crucian carp (*Carassius auratus*), which is palatable and economically important in China (Ji et al., 2010). The species of AsIII and AsV in an environmentally relevant As concentration (50 μg/g) were used (Chai et al., 2016). We chose to concentrate on the fish muscle because dietborne iAs was easily accumulated in the body of crucian carp and muscle accounted for more than 90% of the whole-body burden (Cui et al., 2020). Furthermore, muscle is the edible part for humans and the yield of crucian carp accounts for 15% of the total fish production in China; therefore, accumulated As could be an important dietary risk (Deng et al., 2012). In the present study, we examined whether different dietary iAs species influenced the As bioaccumulation and biotransformation in fish. To profile the temporal metabolisms of iAs, the As accumulation and As species in fish muscle at different exposure times were determined. Additionally, a toxicokinetic study was implemented to reveal the kinetic mechanisms of dietary iAs. This method could provide useful information to identify the adaptation effects on the metabolism of dietary iAs, including influx, biotransformation, and efflux. Additionally, human exposure was also assessed based on the residual iAs levels in muscle of fish under chronic As dietborne exposure.

## 1. Materials and methods

### 1.1. Fish muscle samples

Samples of fish muscle were collected during a 40-day feeding study. A total of 210 fish (13.8–15.5 cm in length, purchased from a fishery in Changsha, China) with mixed sexes were chosen carefully. The fish were randomly divided into three treatments (control, 50 μg/g AsIII treatment, and 50 μg/g AsV treatment). The fish in each treatment were further distributed into seven tanks (50 L dechlorinated tapwater in each tank) with 10 fish per tank. The controls were fed an artificial puffed pellet diet (purchased from Huize Biological Technology Co. Ltd., Hubei, China). The fish in the AsIII and AsV treatments were fed the puffed pellet diet supplemented with AsIII and AsV, respectively. The diets were prepared by adding aqueous solution of arsenate ($Na_2HAsO_4 \bullet 7H_2O$, O2SI smart solutions, Charleston, USA) and arsenite ($NaAsO_2$, O2SI smart solutions) into the pellets, respectively. After the absorption was completed for approximately 5 min, the pellets were lyophilized to a constant weight and stored at -20°C until use.

Fish were provided a suitable environment with the temperature maintained consistently at 24°C and a light/dark cycle of 12 hr. Diet was offered three times per day at 1 g per 100 g fish. Because crucian carp is benthic fish, acclimation is required to train them to feed on floating puffed pellet diets utilized in research. To allow training on battle for the floating diet, fish were acclimatized for approximately 14 days before experimentation. To prevent any waterborne As exposure, any uneaten food was removed from the tank, and the tank water was changed every 24 hr. On days 0, 5, 10, 15, 20, 30, and 40, fish ($n$=7) were weighed and euthanized. Then, the muscle of the whole body was collected and lyophilized to constant weight and stored at -20°C for further analyses.

All efforts were made to minimize sufferings of the fish and numbers of fish utilized. Fish experiments were conducted as prescribed by the guidelines of the Animal Ethical Committee for Animal Experimentation in China.

### 1.2. Determination of arsenic species and total arsenic

As species in ca. 0.2 g of freeze-dried (freeze drier, Lab-1A-50E, Beijing Boyikang Experimental Instrument Co., Beijing, China) fish muscle powder were extracted using a microwave extraction method (MDS-6G, Sineo Microwave Chemistry Technology Co. Ltd., Shanghai, China), and each extracted solution was analyzed using an anion exchange high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC-ICPMS) (Jia et al., 2018b). Various arsenic species (AsIII, AsV, MMA, DMA, AsB, and AsC) were identified

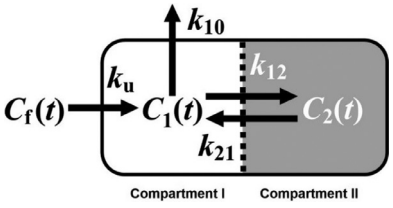


**Fig. 1 – Schematic of the two-compartment model to simulate the dietary arsenite (AsIII) and arsenate (AsV) accumulation in the freshwater food fish, crucian carp (*Carassius auratus*).**

using HPLC (Agilent 1260, Tokyo, Japan) separation with subsequent quantification by hyphenated ICPMS (Agilent 7700x, Tokyo, Japan). The operating parameters for ICP-MS and HPLC followed the description by Jia et al. (2018a). The method detection limit (MDL), obtained according to the U.S. EPA (2011a) using seven replicate analyses of method blanks, was 2 μg/kg for AsC, 3 μg/kg for AsB, 3 μg/kg for AsIII, 3 μg/kg for DMA, 1 μg/kg for MMA, and 9 μg/kg for AsV. The method blank was performed for each batch throughout the entire sample preparation. Standard reference material (SRM) BCR627 Tuna Fish Tissue (Institute for Reference Materials and Measurements, Belgium) was used to estimate the performances of the digestion and analytical methods. The recovery was 86.5%–106.1% for DMA and 92.2%–103.4% for AsB in SRM. Because there was no SRM for fish muscle certified for the other four As species (AsC, AsIII, MMA, and AsV), an in-house reference sample was prepared by adding 5 μg/L As standard mixture to a fish muscle sample containing a trace amount of As. The recovery was 82.8%–106.5% for AsC, 90.5%–101.6% for AsIII, 79.8%–96.5% for MMA, and 88.4%–99.8% for AsV.

For total arsenic analysis, the microwave digestion system was applied to digest fish muscle samples (Cui et al., 2020). Each digested solution was analyzed for total As concentration using ICPMS. The MDL for total As was determined to be 3 μg/kg. The performance of the digestion and analytical methods was estimated using SRM BCR627. The recovery for total As was 88.1%–105.3%. The method blank was performed for each batch throughout the entire sample preparation. The As species and total As in basal and As-supplemented artificial diets were also analyzed and the concentrations/fractions are shown in Appendix A Table S1.

### 1.3. *Arsenic toxicokinetics based on a two-compartment model*

A two-compartment dynamic model was used to simulate the accumulation of As in fish (Fig. 1) (Tan and Wang, 2012; Chen et al., 2018). The dynamic processes include As influx from food, the transfer of As between compartment I and compartment II, As efflux from compartment I, and growth dilution of As. In this model, ingested As was divided into metabolically available and detoxified parts, which were quantitatively separated into compartment I and compartment II, respectively. As was assumed to be accumulated by compartment I from food and then transferred to compartment II. The efflux of As occurred from compartment I. The following Eqs. (1)–(3) were used to explain the parameters involved in the two-compartment toxicokinetic model:

$$C_{int}(t) = C_1(t) + C_2(t) \quad (1)$$

$$dC_1(t)/dt = k_u \times C_f(t) - (k_{10} + k_{12}) \times C_1(t) + k_{21} \times C_2(t) \quad (2)$$

$$dC_2(t)/dt = k_{12} \times C_1(t) - k_{21} \times C_2(t) \quad (3)$$

where, $C_{int}(t)$ (μg/g) is the whole-body As concentration at time $t$ (day); $C_1(t)$ (μg/g) and $C_2(t)$ (μg/g) are the As concentrations distributed in compartment I and II, respectively. Bodyweight changes were not apparent in this study, and thus, growth dilution of As was negligible. The $k_u$ (g/(g•day)) refers to the uptake rate constant; $C_f$ (μg/g) is As concentration in food; $k_{10}$ ($day^{-1}$) is the As efflux rate constant from compartment I; and $k_{21}$ ($day^{-1}$) and $k_{12}$ ($day^{-1}$) are the rate constants for As being transferred from compartment II to I and I to II, respectively. The model fit information is shown in Appendix A Table S2. Both of the $R^2$ values were $> 0.85$ and both of the $p$-values were $< 0.05$, thereby indicating that the model was robust.

Under the equilibrium state, i.e., $dC_1(t)/dt = dC_2(t)/dt = 0$, according to Eq. (3), the fraction of whole-body As distributed in compartment II ($fc_{II}$) can be calculated as Eq. (4):

$$fc_{II} = k_{12}/(k_{12} + k_{21}) \quad (4)$$

The toxicokinetic parameters were calculated by two-compartmental analysis employing Drug and Statistics (DAS) 3.0 software (Mathematical Pharmacology Professional Committee of China, China).

### 1.4. *Human exposure assessment*

The estimated daily intake (EDI, μg/kg average body weight (bw)/day) of iAs ($EDI_{iAs}$) by humans via consumption of fish was calculated by Eq. (5):

$$EDI_{iAs} = C_{iAs} \times dC_{fish}/bw \quad (5)$$

where, the $C_{iAs}$ (μg/g wet weight, ww) indicates iAs concentration in fish muscle, $dC_{fish}$ (g/day) indicates daily consumption of fish, and bw (kg) indicates average body weight of the target population. A dry/wet weight conversion factor of 0.3 was used according to Jia et al. (2018a).

### 1.5. *Statistical analysis*

SPSS (version 19.0; IBM Corp, Armonk, USA) was applied for statistical analysis. A one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test was used to analyze the effect of exposure time or iAs treatment on the concentration of total As and As species in fish muscle. For all of the tests, $p < 0.05$ was regarded as statistically significant.

## 2. Results

### 2.1. *Arsenic bioaccumulation*

During exposure, the dietary and swimming behavior of the fish were normal, suggesting that the health of the fish was not affected by any treatment. Fig. 2 shows the bioaccumulation of total As in fish muscle at different exposure times. As concentration in the fish in the control group remained stable and as low as (0.160 ± 0.050) μg/g. A reduced/balanced As accumulation in fish was detected in both As treatments. In the AsIII treatment, the total As concentrations increased gradually and reached a peak value of (0.457 ± 0.087) μg/g at 30 days, and then declined during the last 10 days. In the AsV treatment, the total As level increased rapidly and reached a peak value of (1.543 ± 0.286) μg/g at day 10, which was 3.4 times

**Table 1 – Toxicokinetic parameters after administration of As-supplemented diets in crucian carp.**

| Treatment | $k_u$ (g/(g·day)) | $k_{12}$ ($day^{-1}$) | $k_{21}$ ($day^{-1}$) | $k_{10}$ ($day^{-1}$) | $fc_{II}$ |
|---|---|---|---|---|---|
| AsIII treatment | 0.041 | 0.678 | 0.029 | 0.708 | 0.990 |
| AsV treatment | 1.521 | 6.913 | 0.091 | 7.293 | 0.959 |

$k_u$ is the uptake rate constant of As; $k_{21}$ and $k_{12}$ are the transfer rate constants of As from compartment II to compartment I and compartment I to compartment II, respectively; $k_{10}$ is the efflux rate constant of As from compartment I; $fc_{II}$ is the As fraction in the whole body assigned to compartment II. See Fig. 1 for the meanings of the parameters.

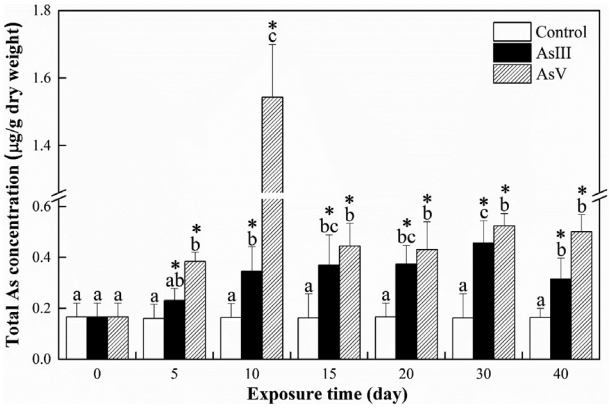


**Fig. 2 – The concentrations of total As in the muscle of crucian carp exposed to 50 μg/g AsIII or AsV. Data are shown as means ± standard deviation ($n = 7$). Asterisks (*) represent significant differences ($p < 0.05$) between the control and the As-exposure treatments at the same time point; different letters indicate significant differences at different times in the same treatments ($p < 0.05$).**

higher than that in the AsIII treatment. The value then decreased rapidly and remained constant during the following 25 days. At day 40, (0.310 ± 0.080) μg/g and (0.501 ± 0.067) μg/g of As remained in fish exposed to AsIII and AsV treatments, respectively. In general, fish accumulated higher levels of total As in muscle in the AsV treatments than in the AsIII treatments.

### 2.2. *Arsenic toxicokinetics based on the two-compartment model*

As shown in Table 1, the toxicokinetics of As in fish were substantially different between the AsIII and AsV treatments. The $k_u$, $k_{12}$, $k_{21}$, and $k_{10}$ were remarkably higher in the AsV treatment than in the AsIII treatment. This indicated that dietary AsV had a faster transfer rate in the fish body than did AsIII. The sum of $k_{12}$ and $k_{10}$, which reflected the capability of the fish to cope with As toxicity by detoxification, was remarkably lower in the AsIII treatment than that in the AsV treatment. The value of $k_{10}$ and $k_{12}$ in both the treatments were nearly equal, suggesting that the efflux and biotransformation of As played equal roles in coping with iAs toxicity. When As bioaccumulation reached equilibrium, the value of $fc_{II}$ was calculated based on the two toxicokinetic parameters, i.e., $k_{12}$ and $k_{21}$. As shown in Table 1, $fc_{II}$ was 99.0% and 95.9% for AsV and AsIII, respectively.

### 2.3. *Temporal variations of arsenic metabolites*

Temporal accumulation and fraction profiles of individual As species are shown in Figs. 3 and 4, respectively. AsB was the only speciation detected in the muscle of the control fish, with a concentration of (0.161 ± 0.044) μg/g, and the other five As species were not detectable. According to the results, the effects of chronic dietary As exposure was significant as the exposure time increased. Additionally, the concentrations of AsIII and AsV in both exposure treatments changed over time with similar trends. In the AsIII treatment, AsIII concentration and fraction initially increased and reached a maximum at day 10 (0.135 ± 0.032 μg/g and 31.8% ± 5.2%), and then decreased gradually from day 10 to day 40. In the AsV treatment, the AsIII species increased faster than in the AsIII treatment, reaching the peak value also at day 10, but with the concentration and fraction as high as (1.257 ± 0.406) μg/g and (79.3 ± 6.4)%, respectively. This was followed by a rapid decrease of the AsIII fraction and concentration from day 10 to day 15, suggesting fast biotransformation or elimination of AsIII in the fish body. The AsIII levels reached equilibrium at day 15 and remained constant during the last 25 days. At the end of exposure, the concentration of AsIII in the AsV treatment was 10-times higher than that in the AsIII treatment. For the AsV species in AsIII and AsV treatments, the concentrations and fractions both increased with increasing time and reached the highest values (0.025 ± 0.006 and 0.076 ± 0.009 μg/g, respectively) at day 15–20, and then decreased rapidly during the remaining days.

Only small fractions of MMA, DMA, and AsC were found in both AsIII and AsV treatments (Fig. 4). MMA was initially detected at day 20, later than DMA. This indicated that being the methylate of MMA, DMA was more easily distributed in fish muscle. The concentrations of AsB species were significantly higher in AsIII and AsV treatments than the control group, with concentrations of 0.276 and 0.353 μg/g, respectively, at the end of exposure. AsB was the main species in both As treatments during the entire period of exposure (except for day 10 in the AsV treatment), with the fractions ranging from 54.0% to 92.4%. Furthermore, the AsB fraction in the AsIII treatment was higher than that in the AsV treatment throughout the exposure period.

### 2.4. *Human exposure assessment*

Because the iAs species (AsIII and AsV) are far more toxic than organic As species (e.g., MMA, DMA, AsC, AsB), the U.S. EPA (2007) utilizes iAs concentration in food to assess potential health risks to humans (U.S. EPA, 2007). Herein, the concentrations of iAs in fish muscle were used to evaluate the health risks of consumption of As-exposed fish to Chinese people according to Eq. (5). Assuming 250 g fish muscle consumed daily and the average Chinese body weight of 58.1 kg (Gu et al., 2006), the EDI of iAs from consumed fish is shown in Table 2. For iAs, the reference dose (Rfd) of 0.3 μg/(kg bw•day) was estab-

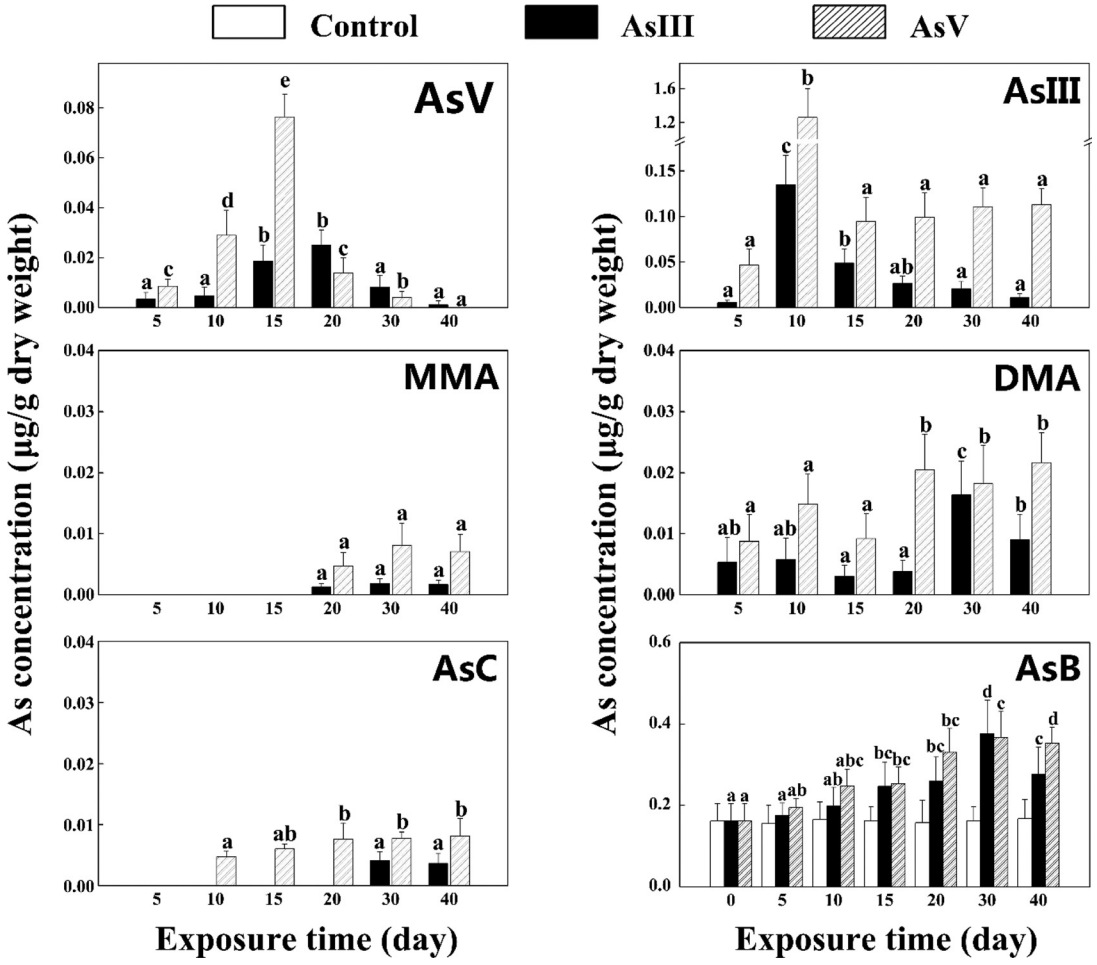


**Fig. 3 – The concentrations of different As species in the muscle of crucian carp exposed to 50 μg/g AsIII or AsV. Data are shown as means ± standard deviation ($n = 7$). Different letters indicate significant differences ($p < 0.05$) at different times in the same treatments. MMA: monomethylarsonate; DMA: dimethylarsinate; AsC: arsenocholine; AsB: arsenobetaine.**

**Table 2 – The concentration of iAs species (inorganic arsenic, AsIII + AsV) in fish muscle after AsIII or AsV exposure treatments for 5, 10, 15, 20, 30, and 40 days, and the corresponding EDI of iAs through fish consumption by the people in China.**

| | AsIII-fed fish | | | | | | AsV-fed fish | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exposure time (day) | 5 | 10 | 15 | 20 | 30 | 40 | 5 | 10 | 15 | 20 | 30 | 40 |
| Concentration of iAs (μg/g ww) | 0.003 | 0.046 | 0.023 | 0.017 | 0.009 | 0.004 | 0.018 | 0.429 | 0.057 | 0.037 | 0.038 | 0.037 |
| EDI (μg/(kg bw•day)) | 0.013 | 0.199 | 0.097 | 0.074 | 0.041 | 0.017 | 0.079 | 1.845 | 0.245 | 0.162 | 0.164 | 0.162 |

EDI: estimated daily intake.

lished by the U.S. EPA (2011b). An EDI that exceeds the R*f*d signifies a health risk to humans. In our study, the risk assessment results indicated that EDI of iAs in the AsIII treatments were <0.3 μg/(kg bw•day) during the 40-day exposure period. Therefore, the crucian carp collected from AsIII (50 μg/g)-contaminated habitats could be considered safe for human consumption. In the AsV treatment, the EDI of iAs from fish sampled at day 10 was 1.845 μg/(kg bw•day), which was 6-times the R*f*d and exhibited an obvious risk in the human diet. However, the exposure time that exceeds 15 days was indicated as safe. The final residues of iAs in the fish muscle of the AsIII and AsV treatment (Table 2) were 0.004 and 0.037 μg/g ww, respectively, after 40 days of exposure. Compared with the peak levels at day 10, the residual iAs amounts in the two treatments dissipated by approximately 91% by the end of the exposure period.

## 3. Discussion

### 3.1. *Arsenic bioaccumulation and toxicokinetics*

The present study demonstrated that the bioaccumulation of iAs in fish muscle is highly associated with exposure

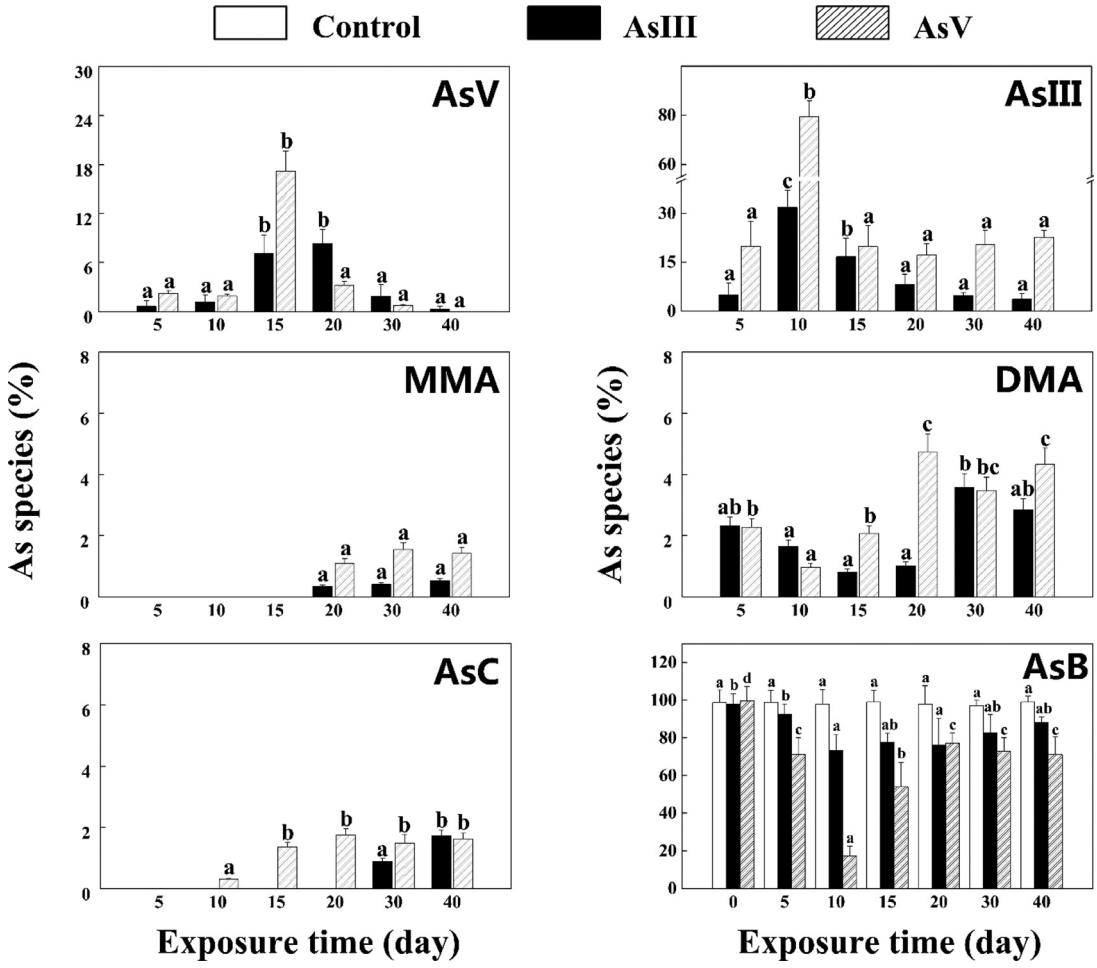


**Fig. 4 – The fractions of different As species in the muscle of crucian carp exposed to 50 μg/g AsIII or AsV. Data are shown as means ± standard deviation ($n = 7$). Different letters indicate significant differences ($p < 0.05$) at different times in the same treatments.**

time, and the adaptation strategy was triggered in fish to cope with As toxicity. Similar results related to the adaptation of fish to exposure of iAs were previously reported. Chen et al. (2018) found that the body As levels in medaka, *Oryzias mekongensis*, increased during the initial 21 days, and then significantly decreased after a week of exposure to waterborne iAs (100 μg/L). Pedlar and Klaverkamp (2002) reported that As accumulations increased in the liver, kidney, stomach, intestine, and pyloric caeca during the initial 30 days but decreased notably after 64 days of dietary exposure in whitefish, *Coregonus clupeaformis*, which was fed 100 μg/g arsenate. All of these results suggest that fish have a strategy to adapt to environmental iAs exposure.

Previous studies showed that glutathione (GSH), glutathione-S-transferase (GST), and two families of transport proteins, aquaglyceroporins (AQP), and multidrug resistance-associated proteins (MRP) could aid in reducing cellular As concentrations in organisms. GSH could elevate As efflux through the formation of a GSH-As complex, which is easily excreted from the body (Aborode et al., 2016; Wang et al., 1996). Additionally, GSH could act as a cofactor for iAs methylation and affect subsequent As elimination (Csanaky et al., 2003). GST is composed of a large family of GSH-utilizing enzymes and plays a vital role in developing As tolerance. As observed in mammalian systems, increased GST activity was associated with elevated hepatobiliary excretion of As (Gyurasics et al., 2015; Wang et al., 1996). The levels of GSH and GST were inhibited in the earlier stage of As exposure in fish kidneys; however, with prolonged exposure, their activity was elevated, suggesting that fish undergo an adaptation to detoxify iAs (Allen and Rana, 2004b; Allen et al., 2004). MRP and AQP contribute to the mediation of As export and uptake, respectively, in killifish (*Fundulus heteroclitus*) (Jung et al., 2015; Miller et al., 2007). However, the overexpression of GST could act as an inhibitor of MRP, and thus, weaken As resistance (Liu et al., 2001; Whaley-Martin et al., 2012). Further investigations are required to elucidate these mechanisms in more detail.

Regulating effects of the adaptation of the accumulation of dietary AsIII and AsV were markedly different in the present study. To provide further insights into the difference between AsIII and AsV treatments, toxicokinetics in a two-compartment model was used to compare the As bioaccumulation processes between AsIII and AsV treatments (Tan and Wang, 2012). Our simulated results showed that the transfer rate constant in the AsV treatment was higher than that in the AsIII treatment. In both AsIII and AsV treatments, biotransformation and efflux played equal roles in coping with iAs toxicity. Approximately 48.9% and 48.6% (i.e., $k_{12}/(k_{12} + k_{10}) \times 100\%$) of newly entered AsIII and AsV, respectively, were transferred

to compartment II, which were much higher than those reported in marine medaka (*Oryzias melastigma*) through waterborne As exposure, where only 14.3% and 2.8% were found, respectively (Chen et al., 2019). Except for the fish species, the exposure pathway could result in this difference. It is worth noting that fish treated with AsV accumulated more As than did those in the AsIII treatment and dietary AsV had a higher rate ($k_u$) to pass into the fish body and be restored in muscle tissue than did AsIII. The difference might be attributed to the different absorbent processes of AsIII and AsV in the digestive tract. Whaley-Martin et al. (2012) reported that AsIII easily sequestered in the digestive gland by binding with thiol moieties of proteins, whereas AsV easily penetrated the wall of the digestive gland. Our results showed that the toxicokinetics of dietary AsIII and AsV in fish were significantly different, which provided useful information for the elucidation of the mechanism involved in the resistance of fish to dietary iAs exposure.

### 3.2. *Arsenic biotransformation*

Our findings confirmed the significance of exposed iAs species and exposure duration in regard to the accumulated As species in fish muscle. Dietary AsV showed a high potential to be transformed into AsIII species and distributed in fish muscle. The AsIII species in AsV treatments were much higher than the directly absorbed AsIII species in the AsIII treatment. The high reduction potential and the subsequent delivery ability of AsV probably contributed to the high capability of As accumulation in the AsV treatment. However, at the end of exposure, AsV was not detectable in the AsV treatment. A possible explanation could be that a substantial portion of assimilated AsV was reduced to AsIII before it was distributed in the fish muscle at the end of exposure (Radabaugh and Aposhian, 2000). Similarly, Francesconi et al. (1989) observed that yelloweye mullet, *Aldrichetta forsteri*, had low retention of AsV in the muscle tissues after being fed a range of As compounds. The reduction of AsV to AsIII was conducted as a detoxification process in the organisms (Allen and Rana, 2004a; Kalantzi et al., 2017). Although AsIII is more toxic than AsV, processes including binding with peptides, transforming into organic As, and expelling from cells, could all weaken AsIII toxicity. After 40 days of exposure, when the steady-state was reached, the AsB (non-toxic and stable As species) fraction was 87.9% and 70.4% for the AsIII and AsV treatment (Fig. 4), respectively, which was less than the respective $fc_{II}$. The reason might be that the minority of As was detoxified in another way, *e.g.*, binding to metallothionein-like proteins (Zhang et al., 2015). The dietary As mainly existed as AsB at the steady-state of bioaccumulation. Our study also demonstrated that the potential of AsIII biotransformation to AsB was higher than that of AsV, based on the greater fraction of AsB in the AsIII treatment at the end of the exposure period. The proportions of AsB were increased in both the treatments with prolonged exposure. This implied an adaptive response in fish by increasing iAs biotransformation into the non-toxic form. Lanno and Dixon (1996) reported that with prolonged exposure to dietary As, the hepatobiliary system of rainbow trout must undergo an adaptation to allow epithelial cells to regenerate. In our study, the levels of each arsenic species could be notably influenced by the adaptation strategies of fish. Considering that the As species of AsIII and AsV are mainly present in the sediment of contaminated habitat, the observation of different As species levels in muscle of wild-caught benthic fish found in previous research may be explained by different dietborne iAs species and exposure duration (Cullen and Reimer, 2010; Jia et al., 2018a).

### 3.3. *Human exposure assessment*

After dietary exposure, the iAs species (AsIII and AsV) were found present in fish muscle. In our results, iAs species from dietary AsV were more prone to distribute into muscle and remain there than did those from dietary AsIII. Consistently, Zhang et al. (2016b) reported that concentrations of iAs in the muscle of AsV-fed marine seabass were nearly two times higher than that in the AsIII-fed group after 28 days of exposure. Similar results were also obtained in marine rabbitfish exposed to 400 μg/g AsIII and AsV (Zhang et al., 2016a). The fractions of iAs in crucian carp were 3.7%–31.8% and 17.2%–79.3% in the AsIII and AsV treatments, respectively, during the 40 days of exposure. It varied substantially at different sampling time-points and with different iAs exposure treatment. Therefore, iAs accumulation in fish muscle is greatly influenced by dietary As species and exposure time. However, in some previous research, 10% of the total As was utilized to represent the iAs fraction when assessing human health risk (Zhang et al., 2018). As a consequence, the health risk might be greatly underestimated or overestimated. In considering the aging processes of fish that can further reduce As residues in fish under dietary iAs exposure, we recommend an interval of 15-day between AsV pollution and fish harvest to ensure human diet safety. Furthermore, it must be noted that different exposure conditions or fish species might show a different kinetic spectrum of total As or As speciation. Therefore, more laboratory studies are needed to address questions of As bioavailability in food fish and facilitate risk assessment for human safety.

## 4. Conclusions

This study examined the temporal concentration and fraction profiles of total As and As species in the muscle of AsIII- or AsV-fed crucian carp. We found that the differential assimilation, detoxification, and efflux capacity of AsIII and AsV contributed to their different bioaccumulation, levels of residual As species in muscle, and the adaptation abilities of fish. The produced AsIII, considered more toxic, was largely formed in the fish muscle at the earlier stage of AsV exposure, which implies increasing toxicity of AsV by crucian carp before consumption by humans. Further experiments are needed to examine the temporal variations of accumulation and biotransformation of As in other benthic fish exposed to dietary As in the future.

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## Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.07.005.

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