Tissue-specific bioaccumulation and health risks of bisphenols in wild fish from West and North Rivers, South China

Yue-Hong Liu¹,², Jun-Wei Huang¹, Zheng Huang¹, Yu-Xian Mei¹, Jian-Liang Zhao¹,², Guang-Guo Ying¹,²

¹SCNU Environmental Research Institute, Guangdong Provincial Key Laboratory of Chemical Pollution and Environmental Safety & MOE Key Laboratory of Theoretical Chemistry of Environment, South China Normal University, Guangzhou 510006, Guangdong, China.
²School of Environment, South China Normal University, Guangzhou 510006, Guangdong, China.

Correspondence to: Prof. Jian-Liang Zhao, School of Environment, Guangdong Provincial Key Laboratory of Chemical Pollution and Environmental Safety, South China Normal University, No. 378, Waihuanxi Road, Panyu District, Guangzhou 510006, Guangdong, China. E-mail: jianliang.zhao@m.scnu.edu.cn


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Abstract
Bisphenols (BPs) are endocrine disruptors (EDCs) that produce hormone effects and other toxic effects. Due to their widespread use, BPs enter into the environment, such as rivers, and hence may accumulate in aquatic organisms. In this study, we investigated the tissue-specific bioaccumulation of BPs in different wild fish species in the North and West Rivers of the Pearl River system, South China, and assessed the human health risks via fish consumption. Firstly, the pretreatment method for 15 BPs in different fish tissues (muscle, liver, bile, plasma, intestine, and stomach) was established, and the target BPs were analyzed using ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The total concentration of BPs in surface water was up to 1,530 ng/L. Bisphenol A (BPA), bisphenol F (BPF), and bisphenol F (BPF) were the dominant ingredients. BPs were widely detected in fish tissues. Among them, BPA had the highest detection efficiency and concentration in fish bile up to 17,160 ng/mL. BPAF had the highest detection efficiency and concentration of 8 ng/g ww in fish plasma. Liver and intestine...
showed log bioaccumulation factors up to 6.93 L/kg, exhibiting high bioaccumulation ability for BPs into biota. The hazard quotients of human exposure risks of BPA via consumption of fish muscle were in the range of $4.97 \times 10^{-4} - 8.97 \times 10^{-4}$, indicating a low health risk of BPA through daily fish consumption.

**Keywords:** BPs, bioaccumulation, West River, North River, fish tissue

**INTRODUCTION**

EDCs refer to a group of compounds that have hormone-like effects and can interfere with the normal functioning of the endocrine system in humans and animals\cite{1}. BPs are a class of typical EDCs that are widely used in various industrial production and consumer goods\cite{2}. For example, BPA is widely used in the production of resins such as polycarbonate and epoxy resin, as well as in the use of plasticizers. BPF is used as a substitute for BPA, as well as for producing resins and various additives. BPs are used as a monomer for synthesizing polysulfone resin, as well as in coatings, leather modifiers, dye intermediates, and metal plating brighteners. Due to their wide range of sources, BPs can enter the receiving environment via various pathways\cite{3}. They can be subsequently transformed in the organism, leading to highly toxic effects\cite{4,5}. It is well-known that BPA produces hormone effects and other toxic effects through the mediation of estrogen receptors, androgen receptors, thyroid receptors, and other pathways\cite{6}. Therefore, various BPA analogs have been developed to replace BPA in the market. However, BPA analogs have similar structures to BPA and also display similar endocrine-disrupting effects and high-risk quotient\cite{7-9}.

BPs are detected in various sources such as domestic wastewater, industrial wastewater, and rainfall runoff in various places around the world. The mean concentrations of BPA, BPF, and BPS were up to 90.0 ng/L in influents in two wastewater treatment plants from the USA, and the removal efficiencies were generally below 50% or even displaying negative removal\cite{10}. BPs were reported with mean concentrations of 98.0 ng/L, 9.60 ng/L, and 200 ng/g in influents, effluents, and sludge from wastewater treatment plants (WWTPs), respectively, in India\cite{11}. The mean concentrations of total BPs were 2,060, 238, and 528 ng/g in influents, effluents, and sludge of WWTPs, respectively, in China\cite{12}. Industrial wastewaters are also detected BPA with concentrations up to 9,074 ng/L in the effluent of papermaking plant\cite{13}. Our studies also found the BPs in domestic wastewater and rainfall runoff in South China\cite{14,15}. Due to source discharge, BPs were then detected in receiving rivers\cite{16-18}. For example, the total concentrations of BPs were up to 2,310 ng/L in the Pearl Rivers, China\cite{14}.

At the same time, as the living environment for aquatic organisms, the continuous inflow of pollutants into rivers can not only disrupt the ecological balance but also accumulate in organisms through ingestion, skin penetration, and gill adsorption\cite{19}. Compounds in the edible compartments of aquatic animals can enter humans through food consumption pathways, potentially posing health risks to humans\cite{20}. Previous literature has reported the bioaccumulation of BPA in fish\cite{21}. BPA was found in wild fish with concentrations of 0.28-12.3 ng/g ww in Taiwanese rivers. Even bioconcentration of BPA in mariculture Flounder can reach 430 ng/g, indicating the harm of BPA to fish and humans\cite{22}. However, there is limited knowledge of BPA analogs in fish tissues. Therefore, it is essential to widely investigate the bioaccumulation of BPs in different tissues in wild fish.

The West and North Rivers are the two main tributaries of the Pearl River system., which are located in the Guangdong and Guangxi Provinces, South China. The upper and middle reaches of the West and North Rivers have high vegetation coverage, while the middle reaches have abundant natural resources and are the important economic center. With the continuous development of industry, a large amount of domestic and
industrial wastewater is discharged directly or indirectly into the West and North Rivers\textsuperscript{[23]}. Emerging pollutants, including BPs, may be further bioaccumulate into biota, causing potential health effects to humans. Therefore, the aims of the present study are: (1) to investigate the levels of 15 BPs in surface waters and wild fish from the West and North Rives of the Pearl River system, South China; (2) to discover the bioaccumulation factors in different tissues (bile, liver, plasma, intestine, stomach, and muscle); (3) to evaluate the human exposure risks via the consumption of fish muscle. The results will contribute to the bioaccumulation assessment and risk management of BPs in the future.

**EXPERIMENTAL**

**Chemicals and reagents**

BPA and 14 BPA analogs [bisphenol AF (BPAF), bisphenol B (BPB), bisphenol C (BPC), bisphenol Cl (BPCI), BPF, bisphenol E (BPE), bisphenol G (BPG), bisphenol P (BPP), bisphenol PH (BPPH), BPS, bisphenol Z (BPZ), bisphenol AP (BPAP), bisphenol BP (BPBP), and bisphenol TMC (BPTMC)] were selected as target analytes. BPA was obtained from Supelco (Bellefonte, USA, > 99%), and the other 14 BPs and 5 isotope-labeled internal standards were purchased from AccuStandard (New Haven, USA, > 99%). The basic information on BPs is shown in Supplementary Table 1 in the Supplementary Material. All stock solutions of the target BPs and their internal standards were prepared in methanol at the concentration of 100 mg/L and kept at -20 °C. The work standards were also prepared in methanol by serial dilution with methanol from the stock solutions.

**Sampling**

Sampling events were conducted in July 2018 in the main streams and tributaries of the West and North Rivers of the Pearl River system, South China. The sampling sites in the West and North Rivers are shown in Figure 1. Seven sites (WR1-7) were selected in the West River, and four sites (NR1-4) were selected in the South River. Surface water with three replicates in each site was collected and stored in 1 L amber glass bottles. Immediately after the collection, surface water samples were adjusted to pH 3.0 by adding 4 M H$_2$SO$_4$ and 50 mL methanol (5%, v/v) to prevent microbial activities. Water samples were transported to the laboratory in a 4 °C cooler for the pretreatment.

Wild fish in active condition were collected using fishing nets, and the body weight and body length were measured. After anesthesia with MS-222, fish were dissected and the tissues collected on-site. The fish dissection has received ethical approval from the South China Normal University with reference No. SCNU-ENV-2018-011. A total of 48 fish in all sites were selected, including Yellow croaker, Grass carp, Tilapia, Yellow catfish, Silver carp, Common carp, Smelt, and Silvery pomfret. The basic information about the fish samples is shown in Table 1. The tissue collection of the fish followed the following steps. First, a 2 mL syringe that had been pre-rinsed with 100 µL sodium heparin solution (1,000 U/mL) was used to collect venous blood from the tail of the fish. The blood sample was transferred to a 1.5 mL centrifuge tube containing 100 µL sodium heparin solution and was subjected to centrifugation (14,000 g, 10 min). The upper layer of plasma was collected. Then, using clean dissecting scissors, an incision was made in the abdomen of the fish to remove the bile, which was placed in a 1.5 mL centrifuge tube. Subsequently, the intestine, stomach, liver, and muscle of the fish were cut into pieces, respectively, using dissecting scissors and surgical forceps. All the collected fish tissues were stored in liquid nitrogen and transported to the laboratory to be stored at -80 °C until pretreatment.

**Sample extraction**

**Water samples**

Surface water samples were filtered through 0.7 mm glass microfiber filters (GF/F) and spiked with the internal standard mixture (100 mL of 1 mg/L for each). The filtered samples were subsequently extracted
Table 1. Information for the wild fish collected from the North River and the West River system

<table>
<thead>
<tr>
<th>Rivers</th>
<th>Species</th>
<th>Number</th>
<th>Body length/cm</th>
<th>Weight/kg</th>
<th>Sampling sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>North River</td>
<td>Silvery pomfret (pampus argenteus)</td>
<td>5</td>
<td>21-46</td>
<td>0.31-4.43</td>
<td>NR2, NR3, NR4</td>
</tr>
<tr>
<td></td>
<td>Grass carp (ctenopharyngodon idella)</td>
<td>3</td>
<td>25.5-39</td>
<td>0.43-1.84</td>
<td>NR1, NR3, NR4</td>
</tr>
<tr>
<td></td>
<td>Yellow croaker (larimichthys)</td>
<td>1</td>
<td>43</td>
<td>2.39</td>
<td>NR3</td>
</tr>
<tr>
<td></td>
<td>Common carp (cyprinus carpio)</td>
<td>2</td>
<td>24-25</td>
<td>0.72-0.74</td>
<td>NR1, NR2</td>
</tr>
<tr>
<td></td>
<td>Smelt (osmerus mordax)</td>
<td>1</td>
<td>27.5</td>
<td>0.73</td>
<td>NR4</td>
</tr>
<tr>
<td>West River</td>
<td>Silvery pomfret (pampus argenteus)</td>
<td>4</td>
<td>24-34</td>
<td>0.34-1.21</td>
<td>WR5, WR6</td>
</tr>
<tr>
<td></td>
<td>Grass carp (ctenopharyngodon idella)</td>
<td>6</td>
<td>18-34.5</td>
<td>0.13-1.11</td>
<td>WR1, WR6, WR7</td>
</tr>
<tr>
<td></td>
<td>Yellow croaker (larimichthys)</td>
<td>2</td>
<td>33-34.5</td>
<td>1.02</td>
<td>WR6</td>
</tr>
<tr>
<td></td>
<td>Yellow catfish (tachysurus fulvidraco)</td>
<td>3</td>
<td>15-17</td>
<td>0.09-0.12</td>
<td>WR4</td>
</tr>
<tr>
<td></td>
<td>Common carp (cyprinus carpio)</td>
<td>7</td>
<td>12-29.5</td>
<td>0.08-0.86</td>
<td>WR2, WR3, WR4, WR7</td>
</tr>
<tr>
<td></td>
<td>Silver carp (hypophthalmichthys molitrix)</td>
<td>1</td>
<td>36</td>
<td>1.59</td>
<td>WR3</td>
</tr>
<tr>
<td></td>
<td>Smelt (osmerus mordax)</td>
<td>4</td>
<td>22-28</td>
<td>0.34-0.79</td>
<td>WR5, WR7</td>
</tr>
<tr>
<td></td>
<td>Tilapia (oreochromis niloticus)</td>
<td>9</td>
<td>13-30</td>
<td>0.08-1.2</td>
<td>WR1, WR2, WR3, WR4, WR7</td>
</tr>
</tbody>
</table>

Figure 1. Map of sampling locations in the North and West Rivers.

with the solid phase extraction (SPE) method with a flow rate of 5-10 mL/min using Oasis HLB cartridges (500 mg, 6 mL). Before this, the SPE column was activated by using 10 mL of methanol and 10 mL of Milli-Q water. After the surface water completely passed through the SPE cartridge, the sample bottle was rinsed twice with a 50 mL aqueous solution containing 5% (v/v) methanol, and the rising solution was also passed through the SPE cartridge. The cartridge was then dried under vacuum conditions for about 3 h. The target BPs were eluted with 12 mL ethyl acetate. The eluate was then combined and slowly blown to near dryness with nitrogen gas, and then made up to volume with 1 mL of methanol. After that, the solution was filtered through a 0.22 µm organic phase filter membrane. The filtered sample was quickly transferred to a 2 mL amber vial and stored at -20 °C for further analysis.
Fish muscle, liver, stomach, and intestine

Fish tissue sample was conducted with enzyme hydrolysis before extraction, and the steps were as follows: 0.5 g of liver (or 2.0 g of muscle/intestine/stomach), wet weight (ww), was transferred into a 30 mL polypropylene centrifuge tube, add 30 µL (30 ng) of mixed internal standard that was redissolved in 0.2 mol/L acetic acid-sodium acetate buffer (pH = 5) and 3 mL of acetic acid-sodium acetate buffer. Five steel beads were added into the centrifuge tube and the tissue sample was homogenized thoroughly for 3 min at 3200 g (-4 °C). Then, add 200 µL of 10% (v/v) β-glucuronidase/arylsulfatase working solution, gently shake the sample and incubate at 37 °C for 12 h. After enzyme hydrolysis, add 10 mL of extraction solvent (containing 2%-5% acetic acid in acetonitrile), vortex and mix, add salt reagent (containing 0.1 g MgSO₄ and 1.0 g NaCl), vortex and mix rapidly, and cool on ice. Homogenize the sample again in the centrifuge (3200 g) for 3 min, perform ultrasonic extraction for 10 min, and then centrifuge at 5120 g for 5 min. Three microliters of post-centrifugation supernatant were taken over the EMR tube (3 mL, Agilent) and the extract was collected into a nitrogen-blowing tube. Blow the extract to near dryness under gentle nitrogen, redissolve in 500 µL of methanol: water (1:1, v/v), and filtered by 0.22 µm organic phase filters into a 2 mL amber vial and store at -20 °C for later instrument analysis.

Plasma and bile

Fifty microliters of bile were transferred to a 1.5 mL centrifuge tube. Add 20 µL (10 ng) of mixed internal standard that was redissolved in 0.2 mol/L acetic acid-sodium acetate buffer (pH = 5) and 10 µL of 10% (v/v) β-glucuronidase/arylsulfatase working solution. Then, add 20 µL of acetic acid-sodium acetate buffer, gently shake to mix, and incubate at 37 °C for 12 h. After enzyme hydrolysis, add 500 µL of acetonitrile (containing 1% acetic acid), vortex-mix for 5 min, and centrifuge at 12,000 rpm for 3 min. Take 1 mL of the supernatant and pass it through a d-SPE purification reagent tube (previously added with 5.2 mL of acetonitrile containing 1% (v/v) acetic acid) by gravity flow, collecting the effluent. After no liquid flows out, add 200 µL of acetonitrile containing 1% acetic acid to rinse the wall of the d-SPE purification reagent tube, and collect the effluent again. Shake the d-SPE purification reagent tube up and down quickly for about 20 s, and centrifuge at 3,500 rpm, 10 min, and 25 °C. 3 mL of post-centrifugation supernatant was taken over the EMR tube and the extract was collected into a nitrogen-blowing tube. Blow the extract to near dryness under mild nitrogen, and redissolve in 500 µL of methanol in water (1:1, v/v). Subsequently, the redissolved extract was transferred across the membrane to a 2 mL brown vial containing a lined tube and stored at -20 °C.

The processing of plasma samples refers to that of bile samples. Add 200 µL of plasma to a 1.5 mL centrifuge tube, add 20 µL (10 ng) of mixed internal standard that was redissolved in 0.2 mol/L acetic acid-sodium acetate buffer (pH = 5), and 20 µL of 10% (v/v) β-glucuronidase/arylsulfatase working solution. Gently shake the sample, and incubate at 37 °C for 12 h. After enzyme hydrolysis, add 1 mL of acetonitrile containing 1% acetic acid, vortex-mix for 30 s, and sonicate for 5 min. Centrifuge at 12,000 rpm for 3 min, take 1 mL of the supernatant, and pass it through an EMR tube by gravity flow. Add 500 µL of acetonitrile containing 1% acetic acid to extract the sample in the centrifuge tube again, repeat the ultrasonic centrifugation steps described above, and take out 500 µL of the supernatant into a 10 mL glass test tube. Rinse the wall of the EMR tube with 200 µL of acetonitrile containing 1% acetic acid, collect the effluent again, and then blow dry under mild nitrogen gas. Add 200 µL of methanol-water (1:1, v/v) to redissolve, vortex-mix, transfer to a sample vial with an inner liner, and store at -20 °C.

Instrumental analysis

Ultra-performance liquid chromatography coupled to a Xevo TQ-S triple quadrupole mass spectrometer (UPLC-MS/MS) (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source was used for quantitative analysis of 15 BPs and 5 internal standards in the final extract in the reaction.
monitoring (MRM) mode. The chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) with an online UPLC filter at the column inlet to remove particulate matter from the sample and mobile phase. The column temperature was set at 40 °C, and the sample tray temperature was set at 15 °C. The injection volume was 5 μL. A post-run time of 1.5 min was set to equilibrate the column pressure and prepare for the next sample analysis. The mobile phase for BPs consisted of water containing 0.05% (v/v) ammonia (A) and methanol (B), and the flow rate was 0.5 mL/min with a gradient elution as shown in Supplementary Table 1 in the Supplementary Material. The main parameters of the ion source were set as follows: the operating gas temperature was set at 550 °C, the cone gas flow rate was 150 L/h, and the nebulizer was set at 7 bar. Nitrogen was used as the collision and drying gas. Other UPLC-MS/MS parameters for BPs (such as precursor ions, product ions, retention times, and collision energies) are shown in Supplementary Table 2 in the Supplementary Material.

Quality assurance and quality control
Before experimenting, all glassware is thoroughly cleaned by ultrasonic cleaning and baking at 400 °C for 4 h. As for plastic equipment such as centrifuge tubes and syringes, disposable principles should be strictly followed to ensure the rigor and accuracy of the experiment. In addition, dissection tools were cleaned with organic solvents, then dried at 40 °C, and wrapped in clean aluminum foil for later use.

We have implemented strict quality assurance and control procedures during the sample processing and analysis. To evaluate the background contamination of experimental materials and solvents, we analyzed procedural blank samples simultaneously with each batch of samples. In addition, during the instrument analysis process, we added a calibration standard sample and a pure solvent sample every 15 samples to assess the stability of the instrument using the calibration standard sample and to evaluate the presence of contaminants between samples and background contamination of the instrument using the pure solvent sample. Furthermore, only trace amounts of BPA were detected in the procedural blank samples, which were far below the levels found in the samples.

The recovery of bisphenols from biological samples ranged from 76%-123%. The method detection limits (MDLs) were calculated using a 10-fold signal-to-noise ratio. The MDLs for fish muscle, liver, stomach, and intestine tissues were 0.12-3.1 ng/g, while those for plasma and bile were 0.08-46.4 ng/mL. When calculating the concentration range, median, and mean of the compounds in the tissues, if the data was below the MDLs, 1/2 MDLs were used instead. If the compound concentration was not detected, zero was used instead. For the calculation of detection rates, when the concentration value was greater than zero but less than the MDLs, the data was considered detected, while a concentration value of zero was considered not detected (ND)\textsuperscript{24}.

Bioaccumulation factors
The bioaccumulation factor (BAF) is an important indicator that reflects the degree of accumulation of compounds in organisms\textsuperscript{25}. With the continuous discharge of pollutants, the bioaccumulation of pollutants can be considered an almost steady-state process. The concentrations of compounds in organisms and corresponding living environments (surface water) were used to calculate the BAF of the pollutants in various tissues using the following equation\textsuperscript{26}. For convenience, we calculate the BAF as the logarithm of BAFs.

\[
\text{Log(BAF)} = \log \left( \frac{c}{c_{\text{surface water} \times k}} \right)
\] (1)
where \( c_i \) is the concentration of BPs of muscle, liver, intestine, and stomach, ng/g ww. \( c_{\text{ bile/plasma}} \) is the concentration of BPs of bile and plasma, ng/mL, while \( c_{\text{ surface water}} \) is the concentration of BPs of corresponding surface water, ng/L. The parameter \( k \) is the unit conversion factor, 1,000.

**Human health risk assessment**

To assess the potential risk of human consumption of fish contaminated by BPs, the risk assessment is conducted using the hazard quotient (HQ) method recommended by the US EPA\[27\]. This indicator can be used to determine whether there is a health risk associated with the intake of BPs, with an HQ greater than 1 indicating a risk and an HQ less than 1 indicating no risk. It should be noted that the higher the HQ value, the greater the risk. The following equations are used to calculate HQ\[14\], which takes into account factors such as the amount of fish consumed by people and the level of contaminants in fish.

\[
CDI = c_{\text{muscle}} \times IR \times FI \times EF \times ED (BW \times AT)
\]

\[
HQ = \frac{CDI}{RfD}
\]

where \( CDI \) is the daily exposure concentrations of contaminants, mg/(kgd). \( IR \) is the daily intake of fish, 0.054 kg/d. \( FI \) is the proportion of contaminated food consumed by fish to total food intake, 1. \( EF \) is the Exposure frequency of fish, 350 d/year. \( ED \) is the duration of fish exposure, 30 years. \( BW \) is the weight; the average body weight of adults was 70 kg. \( AT \) is the total human exposure time, 30 years \( \times \) 365 d/year. \( RfD \) is the reference exposure concentration of pollutants, mg/(kgd), using the US EPA recommended reference dose.

**RESULTS AND DISCUSSION**

**Occurrence of bisphenols in surface water**

The concentration of BPs in the surface water of each sampling site is shown in Figure 2 and Supplementary Table 3. The total concentration of BPs was in the range of 0.62-1530 ng/L, exhibiting incomplete purification from WWTPs or direct discharge from different sources. Overall, the contamination levels of BPs in the North River were higher than those in the West River, which was related to the level of urban development. Among the 15 BPs, BPA (25.5-193 ng/L), BPAF (3.5-58.6 ng/L), and BPF (38.7-303 ng/L), with a 100% detection rate, were much greater than other BPs (< 5 ng/L). These results demonstrated that the two BPA analogs (BPAF and BPF) were extensively utilized as a substitute for BPA in recent years, while other analogs have received comparatively less consideration. In this study, the concentrations of BPAF and BPF in the surface water were significantly higher than those in the Pearl River and Dongjiang River, which belong to the same Pearl River system\[14\]. On the contrary, the concentrations of BPA and BPS (maximum 4.56 ng/L) were significantly lower than those reported in the previous literature\[14\].

**Levels of bisphenol in fish tissues**

The levels of BPs in various fish tissues are shown in Figure 3. The median concentration of BPs followed the sequence: liver, bile, stomach > intestine, muscle > plasma. The BPs with higher detection rates and concentrations were BPA, BPF, and BPAF in all tissues, which is consistent with the concentration pattern of BPs in surface water. The compounds with higher detection rates and concentrations in muscle and liver tissues were BPF (ND-6.26 μg/g); in the intestine and stomach tissues were BPA and BPAF with concentration ranges of ND-333 ng/g and ND-149 ng/g, respectively; in bile was BPA with a concentration range of 5.02 ng/mL-17.2 μg/mL; in plasma samples was BPAF with a concentration range of 1.85-8.83 ng/mL.
In fish muscle samples, all the selected 15 BPs were detected. Among them, the detection rates of BPF and BPAF were both 100%, with the highest detected concentrations of 1,309 ng/g ww for BPF (NR3, Tilapia) and 54.6 ng/g ww for BPAF (WR2, Tilapia). The detection rates of BPE and BPZ were the lowest (both 56%). The highest concentration of BPE was 52.1 ng/g ww (WR2, Tilapia) and the highest concentration of BPZ was 45.6 ng/g ww (WR4 site, Yellow catfish). The concentration of BPA was similar to that in the muscle tissue of fish from the Dongjiang River in China[21]. Fish muscle is the primary consumable portion for humans. The bioaccumulation of BPs in fish muscle contributes to the potential risk to human health.

In fish liver samples, 8 out of the 15 target compounds were detected, including BPA, BPF, BPS, BPC, BPZ, BPCI, BPAF, and BPPH. Among them, BPA and BPAF had the highest detection rates of 94% and 98%, respectively. The highest concentration of BPA was 3,080 ng/g ww (WR7 site, Smelt), and the highest concentration of BPAF was 249 ng/g ww (WR4 site, Common carp). The detection rate of BPZ was the lowest at 28%, with the highest concentration of 35.2 ng/g ww (NR4 site, Smelt). The concentration of BPA was similar to the reported median concentration in fish liver from the Dongjiang River in China, but the mean concentration was much lower than that from the Dongjiang River in China (2991 ng/g ww)[21].

In fish bile samples, 12 out of the 15 BPs were detected, including BPA, BPAF, BPB, BPC, BPCI, BPF, BPE, BPG, BPP, BPPH, BPS, and BPZ. The concentration of BPA was significantly higher than that of other substances, with a detection rate of 100%. The highest concentration of BPA was 17,160 ng/mL (WR7 site, grass carp). Compared with the concentration in fish bile from the Dongjiang River in China, the concentration of BPA was much lower than that in fish bile from the Dongjiang River in China (3,671 ng/mL)[21]. In fish plasma samples, 14 out of the 15 target compounds were detected, including BPA, BPAF, BPAP, BPBP, BPB, BPC, BPCI, BPF, BPE, BPG, BPP, BPPH, BPTMC, and BPZ. Except for BPA with a lower detection rate of 23%, the detection rates of other substances were all above 60%. Compared with the concentration in fish plasma from the Dongjiang River in China, the concentration of BPA was much lower than that in fish plasma from the Dongjiang River in China (47.6 ng/mL)[21].

In fish stomach tissue samples, 13 out of the 15 target compounds were detected, including BPA, BPAF, BPAP, BPBP, BPC, BPCI, BPF, BPE, BPG, BPP, BPPH, BPS, and BPZ. The substance with the highest detection rate was BPAF, with the highest concentration of 607 ng/g ww (WR7 site, Smelt). The substance with the lowest detection rate was BPZ, with the highest concentration being 3.83 ng/g ww (WR3 site, Common carp). In fish intestine tissue samples, 12 out of the 15 target compounds were detected, including BPA, BPAF, BPBP, BPC, BPCI, BPF, BPE, BPG, BPP, BPPH, BPS, and BPTMC. The substance with the
Figure 3. Levels of 15 bisphenols in different fish tissues in West and North Rivers. The percentage number below each box is the detection frequency of each chemical.

highest detection rate was BPA, with the highest concentration being 434 ng/g ww (WR5 site, Smelt).

Fish usually ingest exogenous compounds from water through their gills, gastrointestinal tract, and skin\(^{28,29}\). These compounds are distributed to different compartments of the fish body, such as muscle, liver, bile, and other tissues, through blood circulation. The absorption and metabolism of BPs in fish are different. BPA is mainly distributed in bile, indicating that after being ingested by organisms, most BPA can be excreted into the intestine with bile and eventually eliminated from the body through feces. A small amount of BPA is accumulated in the liver, gastrointestinal tract, and muscle tissues. The liver is the main
distribution site of BPF and BPAF, indicating that the clearance rate of these two compounds in the organism is low, and most of them are absorbed and accumulated in muscle and liver tissues.

According to the location of sampling sites, the West River is divided into five parts: Longjiang, Nanliujiang, Hejiang, Luoding River, and Nanshan River. The sampling sites are distributed in the mainstream of the North River. For bile, liver, intestine, and stomach tissues, the average content of BPA is higher in the Nanshan River. In liver and muscle tissues, the average content of BPF is higher in North and Hejiang. By analyzing the sampling sites, the sites with high concentrations in each tissue were mostly located in WR4 and WR7. WR4 is located in Liuzhou, which is the largest industrial city in Guangxi. BPs, as one of the raw materials in the industry, may enter the environment more due to the extensive use of local industry, leading to a higher biological accumulation in fish.\(^{[30,31]}\)

**Bioaccumulation factors in different fish tissues**

For the fish samples collected from the North and West Rivers in the wild, the bioaccumulation factors (BAFs) of target compounds in each tissue are shown in Table 2. When the bioaccumulation factor is greater than 3.7 L/kg, it can be considered that the compound will bioaccumulate in the tissue.\(^{[32]}\)

The bioaccumulation factor of BPs in muscle ranges from 1.12 to 5.95 L/kg. Except for BPA, BPAF, and BPF, the average bioaccumulation factor of most BPs is greater than 3.7 L/kg, in the following order: BPB > BPTMC > BPE > BPBP > BPG > BP > BPA > BPZ > BPS > BPC > BP > BPA > BPF. The results showed that not only BPA but its analogs may threaten humans because fish muscle is the major edible organ.

The bioaccumulation factor of BPs in the liver, stomach, intestine, and bile tissues was from 0.03 to 6.93. The average bioaccumulation factor of most BPs is greater than 3.7 L/kg, indicating the potential harm of BPs to aquatic organisms. The high level of BPs in the stomach, intestine, and bile tissues may be due to the enterohepatic circulation in these tissues.\(^{[33]}\) In addition, the liver is a significant site for digestion and metabolism,\(^{[34]}\) leading to high logBAFs of BPs. BPs may be metabolized in the fish liver, stomach, intestine, and bile. For example, BPA can be metabolized into two major products (glucuronidated and sulfated conjugates).\(^{[35]}\) However, more toxic products may react in aquatic biota and even humans.

The bioaccumulation factor of BPs in plasma ranges from 0.12 to 4.86 L/kg. The average bioaccumulation factor of BPBP, BPB, BPG, and BPTMC is greater than 3.7 L/kg, indicating the potential harm of BPs to aquatic organisms. The high level of BPs in the stomach, intestine, and bile tissues may be due to the enterohepatic circulation in these tissues.\(^{[36]}\) In addition, the liver is a significant site for digestion and metabolism,\(^{[37]}\) leading to high logBAFs of BPs. BPs may be metabolized in the fish liver, stomach, intestine, and bile. For example, BPA can be metabolized into two major products (glucuronidated and sulfated conjugates).\(^{[38]}\) However, more toxic products may react in aquatic biota and even humans.

Some logBAFs vary widely in concentration in the same tissue. For example, the logBAFs of BPS in fish muscle ranged from 1.12 to 5.72 L/kg. Certain compounds can bind to proteins within organisms, forming complexes that can influence their distribution and accumulation in tissues. Protein binding can either increase or decrease the bioavailability of compounds. Besides, there are significant differences in the logBAFs between the liver (1.97-4.94 L/kg) and bile (0.56-3.43 L/kg) liver and bile, indicating the interspecies differences in metabolism. In conclusion, physiological and metabolic differences between different species can lead to variations in the ability of the same compound to accumulate within different organisms.

**Human health risks**

Because knowledge of previous studies of BPA is relatively limited, toxicity is unknown, and relevant data
Table 2. logBAFs (L/kg) of target bisphenols in fish tissues

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Muscle</th>
<th>Liver</th>
<th>Stomach</th>
<th>Intestine</th>
<th>Plasma</th>
<th>Bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>1.27-3.26</td>
<td>2.39-4.61</td>
<td>1.00-3.90</td>
<td>1.79-3.89</td>
<td>/</td>
<td>2.33-5.36</td>
</tr>
<tr>
<td>BPAF</td>
<td>1.54-3.31</td>
<td>2.18-4.12</td>
<td>0.99-3.36</td>
<td>1.09-4.03</td>
<td>2.60-3.82</td>
<td>3.26-3.27</td>
</tr>
<tr>
<td>BPAP</td>
<td>2.93-5.28</td>
<td>/</td>
<td>3.24-4.39</td>
<td>/</td>
<td>3.48</td>
<td>/</td>
</tr>
<tr>
<td>BPB</td>
<td>2.74-5.81</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>3.62-4.18</td>
<td>3.62-4.18</td>
</tr>
<tr>
<td>BPBP</td>
<td>2.73-5.95</td>
<td>/</td>
<td>3.63-5.48</td>
<td>3.32-6.93</td>
<td>4.24-4.49</td>
<td>/</td>
</tr>
<tr>
<td>BPC</td>
<td>2.99-5.10</td>
<td>3.64-5.42</td>
<td>3.03-5.65</td>
<td>3.08-4.44</td>
<td>/</td>
<td>3.33-4.51</td>
</tr>
<tr>
<td>BPCI</td>
<td>3.15-5.37</td>
<td>4.15-6.36</td>
<td>3.50-4.87</td>
<td>3.40-5.36</td>
<td>/</td>
<td>3.96-4.88</td>
</tr>
<tr>
<td>BPE</td>
<td>3.25-5.94</td>
<td>/</td>
<td>3.48-4.22</td>
<td>3.04-3.89</td>
<td>3.20-3.84</td>
<td>3.63-5.36</td>
</tr>
<tr>
<td>BPF</td>
<td>1.54-4.02</td>
<td>1.97-4.94</td>
<td>0.54-2.08</td>
<td>0.03-2.02</td>
<td>0.12-1.01</td>
<td>0.56-3.43</td>
</tr>
<tr>
<td>BPG</td>
<td>3.03-5.59</td>
<td>/</td>
<td>3.54-5.21</td>
<td>3.62-6.02</td>
<td>3.79-4.60</td>
<td>3.79-4.60</td>
</tr>
<tr>
<td>BPTMC</td>
<td>3.19-5.76</td>
<td>/</td>
<td>/</td>
<td>3.37-5.27</td>
<td>4.53-4.86</td>
<td>/</td>
</tr>
<tr>
<td>BPP</td>
<td>2.96-5.46</td>
<td>/</td>
<td>3.28-5.09</td>
<td>3.15-4.85</td>
<td>/</td>
<td>3.90-4.86</td>
</tr>
<tr>
<td>BPPH</td>
<td>2.88-5.20</td>
<td>2.88-5.36</td>
<td>3.39-5.23</td>
<td>2.88-5.78</td>
<td>/</td>
<td>4.20-4.58</td>
</tr>
<tr>
<td>BPS</td>
<td>1.12-5.72</td>
<td>2.42-4.46</td>
<td>1.80-3.95</td>
<td>2.32-4.90</td>
<td>/</td>
<td>2.52-5.45</td>
</tr>
<tr>
<td>BPZ</td>
<td>3.16-5.26</td>
<td>3.79-5.15</td>
<td>3.26-3.83</td>
<td>/</td>
<td>/</td>
<td>4.20-4.36</td>
</tr>
</tbody>
</table>

are missing, we only assessed BPA. According to the highest detected concentration of BPA in fish tissue, the highest risk of human exposure to BPA through the consumption of different wild fish species is shown in Table 3. The range of HQs for human exposure to BPA through consumption of different fish species is $4.97 \times 10^{-4} - 8.97 \times 10^{-4}$. For the seven common fish species, Tilapia, Common carp, Yellow catfish, Grass carp, Silvery pomfret, Silvery pomfret, and Yellow croaker, the results of the independent sample nonparametric test (Kruskal-Wallis test) showed no significant difference between the HQs among these seven fish species ($P > 0.05$). Therefore, the risk of human exposure to pollutants is not directly related to the type of fish consumed.

As the results show, the HQs of all fish species were far less than 1, indicating that BPA in wild fish would not cause health effects on residents in the West River and North River basins through dietary exposure. Previous studies have shown that various environmental pollutants can usually be simultaneously detected in fish\cite{37,38}, but current risk assessments usually only consider the effects of a single pollutant and do not fully consider the synergistic effects of multiple pollutants. At the same time, most compounds can be transformed into other metabolites through biological metabolism, and these metabolites are usually more toxic than the parent compounds\cite{39}.

The risk assessment of EDCs is particularly complex due to various factors. These factors include the complexity of the interactions between compounds and the endocrine system, potential delayed effects, suggested non-monotonic dose-response relationships, a lack of clear effect thresholds, and the potential effects of very low doses\cite{40}. It is crucial to highlight the lack of knowledge concerning the mechanisms of action of EDCs and the relationships between these molecular events (i.e., interactions with hormone receptors) and adverse health effects. However, new insights in toxicology may lead to the identification of potential new hazards (e.g., chemicals), the reevaluation of exposure standards, or the challenging of old notions about risks. Cognitive uncertainty often persists, such as regarding the intrinsic properties or toxicity of chemicals, making it difficult to make definitive statements about whether chemicals pose a health risk\cite{41}. In summary, future health risk assessments should comprehensively consider the overall impact of different compounds and their metabolites on human health.
Table 3. The maximum hazard quotients (HQs) of BPA for the daily consumption of each fish species

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximum HQ</th>
<th>RfD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilapia</td>
<td>6.72 × 10^-4</td>
<td>0.5</td>
</tr>
<tr>
<td>Grass carp</td>
<td>6.21 × 10^-4</td>
<td>0.5</td>
</tr>
<tr>
<td>Common carp</td>
<td>7.69 × 10^-4</td>
<td>0.5</td>
</tr>
<tr>
<td>Smelt</td>
<td>8.06 × 10^-4</td>
<td>0.5</td>
</tr>
<tr>
<td>Yellow catfish</td>
<td>8.97 × 10^-4</td>
<td>0.5</td>
</tr>
<tr>
<td>Yellow croaker</td>
<td>4.97 × 10^-4</td>
<td>0.5</td>
</tr>
<tr>
<td>Silvery pomfret</td>
<td>6.57 × 10^-4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Data from the US EPA.

CONCLUSION

BPs were widely detected in surface water and fish tissues in the West and North Rivers, the Pearl River system, South China. The concentrations of BPs in surface water were up to hundreds of ng/L for a single compound. BPs showed significant bioaccumulation in fish tissues from surrounding water. BPF showed high detection rates and concentrations in muscle and liver samples with concentrations up to a few μg/g. BPA and BPAF showed high concentrations in intestine and stomach samples with concentrations up to hundreds of ng/g. BPA was detected in bile samples up to tens μg/mL, while BPAFs in plasma samples were up to several ng/mL. The logBAFs for most BPs in each tissue are greater than 3.7 L/kg. Liver and intestine showed the log BAFs up to 6.93 L/kg, indicating relatively high bioaccumulation ability for BPs into biota.

The results showed that the distribution and metabolism of BPs in fish are different. The HQs range for human exposure to BPA through fish muscle consumption is below 4.97 × 10^-4, indicating that BPA in wild fish will not cause health effects via dietary exposure.

DECLARATIONS

Authors’ contributions
Visualization, Formal analysis, Writing the original draft: Liu YH
Investigation, Writing the original draft: Huang JW
Data curation: Huang Z
Investigation: Mei YX
Conceptualization, Writing-review & editing, Funding acquisition, Resources, Supervision: Zhao JL
Writing-review & editing, Funding acquisition, Resources, Supervision: Ying GG

Availability of data and materials
Not applicable.

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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
The fish dissection has received ethical approval from the South China Normal University with reference (No. SCNU-ENV-2018-011).
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