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The measurement of bisphenol A and its analogues, perfluorinated compounds in twenty species of freshwater and marine fishes, a time-trend comparison and human health based assessment

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ABSTRACT

Our previous study in 2011 reported the detection of BPA and PFAAs in 20 species of marine and freshwater fishes. With an emerging evidence to suggest the metabolic-disrupting effects of BPA/PFAAs in animals, the present study was aimed to provide a time-trend analysis to determine the current concentrations of PFAAs and BPA in 20 commercially available Hong Kong species of fishes. Since the manufacture and use of BPA is being prohibited in most nations, the introduction of BPA alternatives has recently been incorporated in the markets. Therefore, the concentrations of BPB, BPF and BPS were determined. In the present study, all freshwater and seawater fish samples showed quantified concentrations [> Limit of Quantification (LOQ $<$ 0.5 ng/g)] of BPA. BPF was detected in some marine (yellow seafin, bigeye, goldspotted rabbitfish, snubnose pompano, tongue sole, Bleeker's grouper and orange-spotted grouper) and freshwater fishes (mud carp, crucian carp, tilapia, catfish, mandarin fish, grass carp, grey mullet and spotted snakehead). Two of the compounds, BPS and BPB could only be identified in the marine fishes (snubnose pompano, yellow seafin). In PFAA analysis, PFOA, PFDA, PFOS, PFUdA and PFDoA were found in most of the marine and freshwater fishes. PFOS and PFOA were shown to be the two predominant PFAAs in fishes. On the basis of the measured concentrations of bisphenols, BPs (BPA, BPB, BPF, BPS) and PFAAs, the average daily intake for BPs (20.5–31.5 ng/kg b.w./day) and PFAAs (1.17–1.83 ng/kg b.w./day) were calculated and found to be lower than values of tolerable daily intake (TDI) established in Europe. However, as compared with our previous study in 2011, the present study revealed an approximate 10-fold increase in the concentrations of BPA in the fish samples. Although the hazard ratio of consuming fishes for BPA and PFAA exposure is expected to remain low, possible additive metabolic-disrupting effect of BPA and its analogues as well PFAAs should be taken into consideration for human health risk assessment.

1. Introduction

In the past century, the production of large amounts of synthetic industrial and biomedical chemicals as well as pollutants poses negative impacts to ecological and animal health (Judson et al., 2009). Many of these chemicals have made to benefit the society; however, the extensive use of the chemicals making them to be widely dispersed in our environment. Chemical pollutants are ubiquitous and are dispersed in air, water, soil and even daily commodities. Human exposure is via different routes and processes including inhalation, dermal contact and ingestion, resulting in the accumulation of different contaminants in blood of general populations worldwide (CDC, 2009). Among different routes of the exposure, food consumption contributes over 90% of total lifetime exposure to many chemical contaminants (Brustad et al., 2008). In coastal areas with intensive industrial activities, the released/disposed chemical pollutants into water systems make fishes a source of various environmental toxicants (Dovydaitis, 2008). Apparently, consumption of contaminated fish would increase concentrations of environmental contaminants in animal bodies, which impose health concern in the public. In fact, the presence of environmental chemicals in human blood samples is considered as one of the risk factors contributing to health problems worldwide (Briggs, 2003). This casual association has been established on the basis of the body pollutant burdens with health dysfunction in wildlife, laboratory animal and human studies.

Humans are exposed to complex mixtures of environmental con-

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taminants in lives. Recent epidemiological evidences to re-analyze 41 cross-sectional and 8 prospective studies of diverse populations has established significant associations of a non-persistent endocrine-disrupting chemical (EDC), bisphenol A (BPA), with the risk of developing traits of metabolic syndrome (Song et al., 2016). BPA is listed as an environmental obesogen, acting on estrogen receptors [ERs: ER-α, ER-β and G-protein coupled estrogen receptor] to perturb body metabolism (Casals-Casas and Desvergne, 2011). With the concerns over its potential impact, a few nations have banned on the use of BPA in food packaging products. It prompts to eliminate the use of BPA or to substitute BPA with its analogues (i.e. bisphenol B, bisphenol S, bisphenol F) (Usman and Ahmad, 2016). However, recent studies reported that the substitutes showed similar biological effects as BPA (Delfosse et al., 2014; Chen et al., 2016). Moreover, the use of BPA analogues is currently not regulated and its potential impact to our environment has yet been elucidated. On the other hand, a group of persistent EDCs, perfluoroalkyl acids (PFAAs) have been prioritized in the European research project OBELIX in 2009 as one of the risk factor in the alternation of development programing for metabolic diseases in life (Legler et al., 2011). PFAAs are classified as peroxisome proliferators (Shipley et al., 2004; Desvergne et al., 2009) that activate ligand-dependent transcription factors of the peroxisome proliferator-activated receptor (PPAR) family, which are known to play pivotal roles in the regulation of insulin signaling, glucose/lipid metabolism and the management of metabolic homeostasis (Sugden and Holness, 2008). In fact, converging epidemiological studies have reported that blood or urine concentrations of BPA and PFAAs are positively associated with dysglycemia, insulin resistance, obesity and/or dyslipidemia (Halldorsson et al., 2012; Silver et al., 2011). Yet, the risks that these chemicals pose in human metabolic health have been assessed and recognized.

Our previous studies reported the presence of BPA and PFAAs in 20 species of freshwater and marine fishes (Wei et al., 2011; Zhao et al., 2011) and sediments in the Pearl River Delta (Zhao et al., 2014), implying the exposure risk of the region. In consideration of the emerging evidences of the metabolic-disrupting effects of BPA and PFAAs, the present study was aimed to provide a time-trend analysis of the current concentrations of PFAAs and BPA. In addition, the concentrations of BPA analogues in the fish samples were determined.

2. Materials and methods

2.1. Sample collection

Twenty fish species (10 marine and 10 freshwater) were obtained in 2016 from local markets in Hong Kong, China. Briefly, fishes were wrapped in aluminum foil and stored on ice 0–4 °C during transportation. On arrival at the laboratory, individual fish was dissected immediately. Six different filleted flesh samples were collected from each fish species. Individual sample was homogenized (Polytron, Capitol Scientific, Inc., Austin, United States) and stored in −20 °C freezer until extraction and subjected to BPA with its analogues and PFAA analysis using an Agilent 1200 high-performance liquid chromatograph coupled with tandem mass spectrometry (HPLC-MS/MS, Agilent 1200 series, Agilent Technologies, California, USA). To avoid possible cross-contamination among samples, dissecting kits and homogenizer were washed in Milli-Q water (Millipore, Billerica, United State), followed by methanol for every sample preparation.

2.2. Chemicals and instrumental analysis for BPA with its analogues

Methanol (HPLC grade), acetonitrile (HPLC grade) and chloroform (HPLC grade) were purchased from LABSCAN, UK. Bisphenol S (4,4′-sulfonyldiphenol, BPS) and bisphenol F (bis(4-hydroxyphenyl)methane, BPF) were purchased from Sigma-Aldrich, Germany. Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA) was purchased from AccuStandard, USA. Bisphenol B (2,2-bis(4-hydroxyphenyl)butane, BPB) was purchased from Dr. Ehrenstorfer, Germany and bisphenol A-d16 was purchased from Cambridge Isotope Laboratories, Inc. (CIL), USA. Stock solutions (1000 ng mL^{-1}) of BPS, BPF, BPA, BPB and BPA-d16 were prepared in methanol. Milli-Q water (Millipore, Milli-Q system) was used in sample preparation. All equipment, glassware and polypropylene (PP) centrifuge tubes (IWAKI, Japan) were pre-washed three times by acetone followed by methanol. An Agilent 1200 series liquid chromatography (Waldbronn, Germany), equipped with a quaternary pump (G1311A) and an autosampler (G1329A) was used for LC–MS/MS analysis. Chromatographic separation was performed by using an Agilent ZORBAX Eclipse Plus C8 Narrow Bore guard column (2.1 mm × 12.5 mm, 5 μm) and an Agilent ZORBAX Eclipse Plus C8 Narrow Bore column (2.1 mm × 50 mm, 3.5 μm). Tandem mass detection was conducted by an Agilent 6410B Triple Quadrupole mass spectrometer system equipped with an Agilent Masshunter Workstation (version B.02.01) and an electrospray ionization source. In order to achieve greater sensitivity, analytes were detected in a dynamic Multiple Reaction Monitoring (MRM) mode. The ionization source parameters were as follow: ion spray voltage, −4000 V; source temperature, 350 °C; nebulizer pressure, 25 psi; dry gas flow, 10 L min^{-1}; delta EMV, 900 V for negative. Collision energy (CE) and fragmentor of BPS, BPF, BPA, BPB and BPA-d16 were also optimized to obtain maximum sensitivity (Table 1).

An aliquot of 0.2 g freeze-dried sample (accurate to three significant figures) was extracted. Twenty nanograms of deuterated BPA (BPA-d16) was added to each sample as an internal standard. Each sample was extracted with 10 mL acetonitrile in a pre-washed 50 mL PP centrifuge tube. The sample was extracted in an ultrasonic bath (Models 3510, Branson, USA) for 30 min and was then mixed in a digital reciprocating shaker (HS501, IKA, Germany) for 30 min at 300 mot min^{-1} at room temperature. The solution was centrifuged (Allegra 6R, Beckman, USA) at 1500 rpm for 15 min. The supernatant (the acetonitrile phase) was filtered and saved. The extraction was repeated twice and all the extracts pooled. The extract was dried by a rotary evaporator and re-dissolved in 1 mL of methanol/water (50:50) prior to LC/MS/MS analysis.

Gradient LC conditions were adopted for separation and quantification of bisphenols (BPs) in fish samples. In brief, mobile phases were A: Milli-Q water and B: methanol. A five-step gradient with a flow rate of 0.25 mL min^{-1} was used as follows: 0 min, 50%B; 0–5.5 min, linear gradient from 50%B to 99%B; 5.5–6.5 min, 98%B; 6.5–7 min, linear gradient from 99%B to 50%B; 7–15 min, 50%B. The injection volume was 30 μL and the column temperature was maintained at 30 °C

Table 1
Multiple reaction monitoring (MRM) conditions for identification and quantitation of BPA and its analogues.

Compound	Retention time (min)	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)
BPS	2.116	249	156	135	14
BPF	4.256	199	105	135	16
BPA	7.408	227	212	130	10
BPB	8.237	241	211	130	20
BPA-d16	7.263	241	223	95	12