TISSUE DISTRIBUTION OF BISPHENOL A IN TIPPED REEF SHARK AND RISK ASSESSMENT OF SHARK FIN CONSUMPTION

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Introduction

Bisphenol A (BPA) is a synthetic industrial chemical, mainly used for the production of polycarbonate (PC) plastics and epoxy resins which are widely used in different products of daily life, including digital media (typically CDs and DVDs), electronic equipment, construction glazing, sports safety equipment, medical devices (e.g. dental sealants), tableware, reusable bottles (e.g. baby bottles), and food storage containers (e.g. food cans)[1]. For the wide application of BPA, it has a large worldwide production volume as well as with a rapid growth rate of production demand. The global demand of BPA has increased from 3.9 million tonnes in 2006 to 5 million tonnes in 2010[2]. The global BPA demand is mainly driven by the demand from Asian countries, especially China. The BPA production volume of China had been estimated about 2.25 million tonnes in 2010, accounting for 45% of the global production capacity[3].

BPA is classified as an endocrine disruptor, which may disrupt the reproduction system of wild life and humans by acting as an estrogen agonist[4] or an androgen antagonist[5]. Human exposure routes of BPA include migration from food packages (water bottles, baby bottles, beverage bottles, package papers, food cans), intake of polluted drinking water and food (fish, meat, vegetables), inhalation of ambient and indoor air, etc. Among these routes, dietary intake is believed to be the most important source of BPA exposure for humans[1]. It is thus of great importance to monitor BPA contamination in different kinds of food.

Sharks have been in the oceans as the top predators for more than 400 million years. They have played an important role in the regulation and maintenance of the balance of ocean ecosystems[6]. However, sharks are being threatened by the business of shark fin trade. Shark fin consumption occupies a long history in the Chinese food culture and diet. It is treasured as highly valued food and mainly served on auspicious occasions, such as wedding banquets and business dinners[7]. Having shark fin imported from 116 countries across the globe, Hong Kong, located in Pearl River Delta of China, is the center of shark fin trading. Due to shark finning, currently more than 100 million sharks are taken from the sea each year. By 2017, it is anticipated that 20 species of sharks would become extinct due to indiscriminate fishing techniques[8]. Besides the ecological impact caused by shark finning, consuming shark fin may pose health risks for humans. Man et al.[7] suggested consuming shark fin may result in high non-cancer risks exposure to mercury contained in shark fin through dietary intake. Except for mercury, there are few studies about other pollutants in shark fin. The present study aims to: 1) study the BPA occurrence in different tissues of sharks from the surrounding sea of Pearl River Delta, including fins; 2) assess the potential health risk resulted from shark fin consumption containing BPA.

Materials and methods

Sample collection and preparation

In order to study BPA occurrence in different tissues of shark, five individuals of tipped reef shark (*Carcharhinus melanopterus*) were collected in the sea near Hong Kong with the assistance of local fisherman. They had a similar body length of 77-84 cm and body weight of 2.7-3.6 kg. On arrival at the laboratory, the sharks were dissected with a clean scalpel to obtain the different tissues, including muscle, gill, liver, stomach, intestine, dorsal fin, and tail. Commercial shark fins are usually produced from dorsal fin and tail of sharks, so that dorsal fin and tail were collected for BPA occurrence investigation. All tissues were then freeze-dried, and their water contents were determined. The skin and muscle of the dried dorsal fins and tails were removed. Then all dried samples were ground into fine powder, homogenized, and stored in desiccators until extraction. All the equipment used for sample collection, transportation, and preparation were rinsed with methanol before use to avoid interference.

Reagents
Methanol (HPLC grade), ethanol (HPLC grade), dichloromethane (HPLC grade), and n-hexane (HPLC grade) were purchased from Fisher Chemical. Ultrapure water (Milli-Q system, Millipore) was used for sample preparation. Standards of bisphenol A and bisphenol A-d16 were purchased from AccuStandard.

Sample pretreatment and LC/MS/MS analysis
The sample pretreatment and LC/MS/MS analysis conditions can be found in the previous study[9]. In brief, 0.2 g dried shark tissue was extracted by 10 mL methanol in ultrasonic bath (Branson) and then digital reciprocating shaker (IKA) for twice. The combined organic layer was mixed vigorously with 15 mL hexane to remove lipid. Then the methanol layer was dissolved in 200 mL ultrapure water and purified by solid phase extraction cartridges (Oasis HLB, 6cc, 200 mg, Waters). Samples were separated by Agilent 1200 liquid chromatography equipped with a C8 guard column (Agilent ZORBAX Eclipse XDB-C8, 2.1 mm×12.5 mm, 5 μm) and a C18 column (Agilent ZORBAX Eclipse XDB-C18, 2.1 mm×50 mm, 3.5 μm). Tandem mass detection was conducted by an Agilent 6410B triple quadrupole mass spectrometer system with electrospray ionization source in Multiple Reaction Monitoring (MRM) mode.

Quality control
Three replicates for each sample were analyzed for accuracy. The method detection limit of BPA is 0.1 ng/g dw (dry weight). Recovery of the spiked surrogate standard BPA-d16 ranged from 77-109%. 5 ng BPA-d16 had been added into different kinds of matrices, e.g., muscle, fin, etc., to check the ion suppression of LC/MS/MS, and the ion suppression ratio was controlled within 20%. For each batch of 12 samples, one procedural blank was performed to check the BPA interference during sample analysis, and no interference had been found in the procedural blank samples. One calibration curve with concentration range of 0.1-25 ppb was used for quantification, and analysis of a standard with middle concentration of 2 ppb was performed after every ten samples to check the stability of the equipment.

Statistical analysis
Statistical analysis was performed using SPSS 19.0. Different groups of data were compared by analysis of variance (ANOVA) followed by Duncan's Multiple Range test (significance at $p<0.05$). When BPA concentration was below the detection limit for some samples, half of the detection limit was used for statistical purpose.

Results and discussion

BPA occurrence in different tissues of shark

BPA was detectable in different kinds of tissue samples from investigated sharks. BPA concentration ranges were 0.2-2.3 ng/g ww (wet weight) for muscle, ND (not detected)-0.1 ng/g ww for gill, 0.4-1.4 ng/g ww for liver, ND-1.7 ng/g ww for stomach, ND-0.3 ng/g ww for intestine, 0.5-1.6 ng/g ww for dorsal fin, and 0.1-4.7 ng/g ww for tail. BPA occurrence in sharks can be attributed to the sea water pollution. Pearl River Delta where Hong Kong is located is a highly developed region of China with dense population and industry, which is regarded as a highly polluted region with large discharge amount of municipal and industrial sewage into Pearl River and the receiving marine environment[10]. BPA had been detected in both surface water and sediments of Pearl River and the adjacent sea[11,12]. Although toxicokinetic studies suggest BPA could be metabolized rapidly in biota[13], the occurrence of BPA in investigated sharks indicates the persistent pollution of the marine environment by BPA-containing sewage.

As shown in Fig. 1, among the investigated organs, gill and intestine had the lowest BPA level ($p<0.05$) with average concentration of both 0.1 ng/g ww respectively. Muscle, liver, and stomach had comparable BPA contents (0.6 ng/g ww in average), which was significantly higher than that of gill and intestine ($p<0.05$). Muscle and liver are commonly used for studying the bioaccumulation of organic pollutant in biota, for the affinity of many organic pollutants with the lipid of muscle and the importance of liver for accumulation, biotransformation, and excretion of pollutants[14], while stomach is the major site for digestion of food which may be contaminated. This may explain their relatively higher BPA occurrence. Dorsal fin and tail which are used for shark fin production contained the highest level ($p<0.05$) of BPA, with average concentration of 1.1 and 2.2 ng/g ww respectively. The preferrential accumulation of BPA in dorsal fin and tail suggests the potential health risk of shark fin consumption. Edible part of commercial fin is the cartilage of fin and tail, which is mainly composed of proteins (80%) and also lipids, carbohydrates, and minerals[15]. It is possible BPA has affinity with proteins, which may explain the relatively higher occurrence in fin and tail compared with other tissues. When comparing between dorsal fin and tail, tail contained significantly ($p<0.05$) more BPA than dorsal fin. The highest BPA concentration of 4.7 ng/g ww was also found in a tail sample. BPA level may be related with the size of fin and tail. The investigated tails and dorsal fins have averaged length of 18 and 7 cm respectively. Larger cartilagenous organs probably tend to accumulate more BPA. When comparing within the five shark individuals, there are no significant di butable to their similar size and body

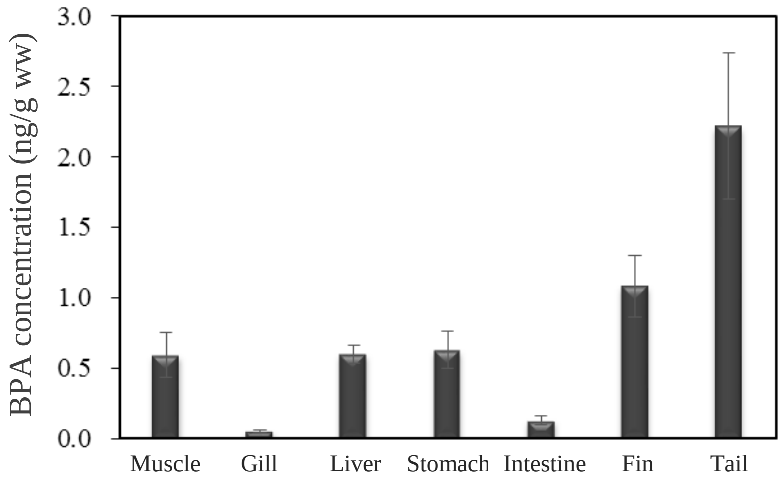

Fig. 1. BPA concentration in different tissues of tipped reef shark

Estimated intake of BPA via shark fin consumption

For the preferrential accumulation of BPA in dorsal fin and tail of sharks, shark fin consumption may pose risks to humans. The potential health risk of BPA-containing shark fin consumption can be calculated by the ratio of estimated daily intake (EDI, μg/kg/day) and a reference dose (RfD, μg/kg/day)[16]:

$$\text{Hazard Quotient (HQ)}=\text{EDI}/\text{RfD} \qquad (1)$$

HQ ≤ 1 suggests no adverse effects, while HQ >1 suggests possible adverse effects.

$$\text{EDI}=(\text{Concentration}\times\text{Consumption rate})/\text{BW} \qquad (2)$$

Where, Concentration: BPA concentration in shark fin (μg/kg)
Consumption rate: shark fin consumption per day (kg/day)
BW: body weight (kg)

According to US EPA[17], RfD for BPA is 50 μg/kg/day. The average BPA concentration in dried dorsal fin and tail is 4.1 ng/g dw (dry weight). Body weight of 60 kg and 15 kg for adults and children are used for Hong Kong population[7]. The average consumption rate of shark fin was estimated according to the total consumed shark fin and population size of Hong Kong as 1.74 g/day for adult[7]. The consumption rate ratio for adults and children is 1.86:1, resulting shark fin consumption rate of 0.96 g/day for children. In an extreme case, shark fin consumption rate can be much higher than the normal condition. A dish called Braised Giant Shark Fin had 4 taels (1 tael=37.5 g) of shark fin for one person. The consumption rates for adults and children are 0.15 and 0.08 kg/day respectively[7] in this case.

Accordingly, for the normal case, average HQ for adults and children are 2.4×10^{-6} and 5.3×10^{-6}. For the extreme case, average HQ for adults and children are 6.5×10^{-4} and 1.4×10^{-3} (Table 1). The much smaller HQ than 1 suggests consumption shark fin, even in the extreme case, may not pose any health risk. However, considering other major BPA intake routes in daily life, e.g. food package, water bottles, beverage bottles, etc., the health risks derived from BPA may not be overlooked.

	Daily consumption of shark fin		Extremely high consumption of shark fin	
	Adult	Child	Adult	Child
Medium	0.0000024	0.0000053	0.00021	0.00044
5th percentile	0.00000017	0.00000038	0.000015	0.000032
95th percentile	0.0000075	0.000017	0.00065	0.0014

Table 1. Hazard Quotient (HQ) of BPA for daily and extremely high consumption of shark fin

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