Front. Environ. Sci. Eng. 2015, 9(3): 475–481
DOI 10.1007/s11783-014-0640-6

RESEARCH ARTICLE

Determination and occurrence of endocrine disrupting compounds, pharmaceuticals and personal care products in fish (*Morone saxatilis*)

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Abstract Endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs) have attracted much attention due to widespread contamination in aquatic environment. In this study, we determined 13 EDCs and PPCPs in fish blood, bile and muscle by using gas chromatography-mass spectrometry (GC-MS). The limits of quantitation (LOQ) were in the ranges of 0.23–2.54, 0.22–2.36 $ng \cdot mL^{-1}$, and 0.24–2.57 $ng \cdot g^{-1}$ dry weight (dw) for fish blood, bile and muscle, respectively. Recoveries of target compounds spiked into sample matrices and passed through the entire analytical procedure ranged from 65% to 95%, from 60% to 92% and from 62% to 91% for blood, bile and muscle, respectively. The methods were applied to the analysis of fish from a lake in California. Target compounds were relatively low in bile, and only bisphenol A (BPA) and diclofenac were measurable near the LOQ. Seven of 13 compounds were detected in blood, with total concentrations up to 39 $ng \cdot mL^{-1}$. Only BPA was frequently found in muscle, with mean concentration of 7.26 $ng \cdot g^{-1}$ dw. The estimated daily intake of BPA through fish consumption for U.S. resident was significantly lower than the tolerable daily intake recommended by the European Food Safety Authority. This study showed that the exposure to the bisphenol A from fish diet is unlikely to pose a health risk.

Keywords endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs), fish, bisphenol A (BPA), risk assessment

1 Introduction

Endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs) are emerging contaminants that have provoked much public and scientific attention [1]. These compounds that were excreted by patients and improperly disposed by the users eventually found their ways into the wastewater treatment plants (WWTPs). Since WWTPs can not completely remove these compounds, the treated effluents of WWTPs were acknowledged as an important source for PPCPs and EDCs to enter the aquatic environment [2,3]. Although they were detected at low concentrations ($ng \cdot L^{-1}$) in recipient waters, continuous release and chronic exposure to these compounds may possess deleterious effects on aquatic mammals and potential risk to human health [4,5].

In our previous study [6], we have found PPCPs and EDCs were widely present in effluent at level of $ng \cdot L^{-1}$ and has been released into the aquatic environment. The accumulation of these chemicals and their potentially adverse effects on wildlife and human is an increasing concern. Fish are the most likely vertebrate to be affected by EDCs and PPCPs in the aquatic environment. A few studies were on measuring PPCPs and EDCs in fish. Gibson et al. [7] found estrogenic contaminants in bile of fish exposed to WWTP effluents. Brown et al. [8] studied the bioconcentration of human pharmaceuticals from sewage effluents into fish blood plasma. Corcoran et al. [9] reviewed the presence and reported biological effects in fish of most commonly detected pharmaceuticals in the aquatic environment.

However, little is known on their occurrence in muscle of fish survived in nature water, which is an important topic, because they may enter food-chain and eventually consumed by human. Brooks et al. [10] provided the first report of PPCPs in muscle tissues of fish residing in a municipal effluent-dominated stream. Ramirez et al. [11] analyzed pharmaceuticals in fish muscle tissues and detected 4 chemicals with concentrations ranged from 0.11 to 5.14 $ng \cdot g^{-1}$. Azzouz et al. [12] extracted estrone,

Received April 27, 2013; accepted September 4, 2013

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17β-estradiol, and florfenicol from fish flesh, with concentrations ranging from 0.52 to 3.4 $ng \cdot g^{-1}$.

In this study, 13 PPCPs and EDCs, distributed among different therapeutic classes (Table S1), namely, analgesics, anti-epileptic, antibiotics, endocrine disruptor, lipid regulators, and non-steroidal anti-inflammatory drug (NSAID) were monitored. We developed a simultaneous extraction and analysis method using gas chromatography–mass spectrometry (GC-MS) to determine the concentrations of 13 chemicals in fish bile, blood and muscle. Further, we investigate the occurrence of them in surface water and fish from a lake in California. Finally, we estimated the daily intake of target compounds and potential health risks associated with fish consumption. To our best of knowledge, this is the first study to document the human exposure and risk to bisphenol A via fish consumption.

2 Experimental section

2.1 Chemicals and reagents

Standards of 13 PPCPs and EDCs are following: 4-*n*-nonylphenol (NP), 4-tert-octylphenol (OP), aspirin (ASP), bisphenol A (BPA), carbamazepine (CBZ), clofibric acid (CFA), diclofenac (DCF), estrone (ETR), gemfibrozil (GFB), ibuprofen (IBP), ketoprofen (KTP), naproxen (NPX) and triclosan (TCS). Chemical structures and physical-chemical properties of the chemicals are shown in Supplementary Materials (Table S1). The surrogate standard, [$^2H_3$]-ibuprofen (D3-IBP), was purchased from C/D/N Isotopes Inc. (Quebec, Canada). Stock standard solutions (1000 $mg \cdot L^{-1}$) were prepared in methanol and stored at −20°C. *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) (Sigma-Aldrich, St. Louis, MO, USA) was used as the derivatizing reagent. Acetone, ethyl acetate, methanol (pesticide grade), formic acid and hydrochloric acid (HCl) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized water was purified by a Milli-Q system. Oasis hydrophilic lipophilic balance (HLB, 150 mg, 3 mL) was purchased from Waters (Milford, MA, USA).

2.2 Sample collection

In September 2011, 52 striped bass (*Morone saxatilis*) were collected from Lake Skinner (33°35′18″N 117°03′24″W), California, which is supplied by the Colorado River Aqueduct and the State Water Project, and feeds a water filtration plant nearby, and in turn, supplies water to many local residents. Fish were collected on Tuesdays to reflect normal activities of community. The approximate size of fish collected were similar and ranged from 1.5 to 2.0 kg and 34 to 40 cm. Samples were frozen on ice and immediately transported to the laboratory. All the samples were stored at −20°C until further analysis. Lake water were sampled at the same time. The water samples passed through glass fiber pre-filters, treated with 0.5% sodium azide (w/v), and stored at 4°C.

2.3 Extraction and analysis

The extraction method is solid phase extraction (SPE). One mL of blood samples were diluted in 50 mL deionized water. The sample was filtered by glass fiber pre-filters and adjusted to pH 2 with HCl (37%) prior to extraction. The HLB cartridges were conditioned with 2 mL methanol and 2 mL acidified deionized water (pH = 2), followed by loading of the sample at a flow rate of 5 $mL \cdot min^{-1}$. The cartridges were dried under nitrogen and eluted with 3 × 1 mL methanol. Two hundred mL of lake water were passed through HLB cartridge as described above.

Fish bile sample preparation and cleanup were performed similar to a method previously published by Gibson et al. [7]. One mL of bile samples diluted with 2 mL 0.2 $mol \cdot L^{-1}$ acetic acid buffer (pH 5.0). The solution was added 40 μL β-glucuronidase and incubated at 37°C for 8 h for the enzymatic hydrolysis of glucuronides present in bile samples. Then they were diluted with 50 mL deionized water, adjusted to pH 2 with HCl, and extracted by HLB as described above.

The defrosted fish muscle were sliced and homogenized in a blender to obtain a fine mash. One gram of prepared muscle sample was mixed with methanol containing 1% (v/v) formic acid, successively vortexed for 2 min, ultrasonicated for 10 min, and centrifuged at 4000 $r \cdot min^{-1}$, then decanted the supernatant. The muscle sample was extracted two additional times with 4 and 3 mL of methanol containing 1% (v/v) formic acid. The supernatants were combined and evaporated by nitrogen to about 1 mL, diluted with 100 mL deionized water, then cleanup by HLB cartridge.

The eluates were evaporated to dryness with a gentle stream of nitrogen at 37°C, and redissolved in 180 μL of ethyl acetate, then transferred into glass insert in the GC vial, and 20 μL of MTBSTFA was added. The GC vials were put into GC oven at 70°C for 60 min for derivatization prior to GC-MS analysis, which can convert polar groups into relatively nonpolar groups. The concentrations of analyzed chemicals were determined by using an Agilent 6890N GC coupled with a 5975C MSD, equipped with an Agilent 7683B automatic liquid sampler (Santa Clara, CA, USA). Details regarding instrumental analysis are given in the Supplementary Materials.

2.4 Quality assurance and quality control (QA/QC)

QA/QC was carried out to ensure the accurate quantification of the target chemicals. The recovery of the method was evaluated by determining samples (triplicates) spiked with known concentrations of analytes (2 and 20 $ng \cdot mL^{-1}$

for bile and blood; 2 and 20 $ng \cdot g^{-1}$ for muscle). The instrumental detection limit (IDL) and instrumental quantification limit (IQL) were set as signal to noise (S/N) ratio $\geqslant 3$ and 10, obtained from serial dilution of standards. The limit of quantification (LOQ) was determined by calculating S/N ratios $\geqslant 10$ of each compound. All the samples were analyzed in duplicate, and all the analytical results are reported as the average of two values.

Quantification was performed using linear regression equations ($R^2 > 0.99$) generated from an eight point calibration standard prepared in ethyl acetate with concentrations spanning from 0.5 to 100 $ng \cdot mL^{-1}$. A constant amount of deuterium labeled surrogate standards (50 ng), D3-IBP, was added before the extraction for compensating the differences in extraction yields between different analytes, and for variations between samples with regard to physico-chemical properties.

3 Results and discussion

3.1 Method performance

Figure 1 show the typical chromatogram for a standard solution (50 $ng \cdot mL^{-1}$) and a fish blood sample. The instrumental and procedural blanks of target compounds were not detectable or far below LOQ. The peak area repeatability obtained from five repeated injections of a spiked sample were lower than 6% (Table S2) from the RSD, reflecting the stability of the equipment.

The extraction and cleanup procedures for the analysis of PPCPs and EDCs in fish muscle were optimized by testing extraction efficiencies of various solvents. We tested extraction efficiencies of acetone, methanol, ethyl acetate, acidified methanol. As shown in Fig.2, acidified methanol provided the greatest recoveries for all target analytes. The recoveries of 13 target compounds in muscle samples were from 62% to 91% (Table 1). The results were similar to those reported by Ramirez et al. [11], where they obtained the best recoveries, from 31% to 97%, by 0.1 mol $\cdot L^{-1}$ acetic acid methanol. Moreover, the LOQ of 13 chemicals were 0.22 to 2.56 $ng \cdot g^{-1}$ (Table 1), suggesting that the method is suitable for trace analysis of these chemicals in fish muscle.

3.2 PPCPs in lake water

The 13 compounds we investigated, except for CFA and KTP, were found in lake water (Table 2). The LOQs of of 13 chemicals in lake water ranged from 0.5 $ng \cdot L^{-1}$ (BPA) to 5.6 $ng \cdot L^{-1}$ (DCF). BPA exhibited the highest concentration of 43.9 $ng \cdot L^{-1}$, followed by NPX and DCF at concentrations of 19.4 and 5.6 $ng \cdot L^{-1}$, respectively. TCS, GFB, IBP and ETR were detected in medium concentration levels with means of 2.2, 1.7, 1.3 and 1.1 $ng \cdot L^{-1}$, respectively. The concentrations of ASP, CBZ, NP and OP were < 1 $ng \cdot L^{-1}$. These values are far lower than predicted no-effect concentrations (PNEC) in previous study [6], indicating no immediate ecological risk is expected. Compare with other countries in the world [13–16], the concentrations of PPCPs and EDCs in lake water are at a minimally contaminated level.

3.3 Analysis of fish samples

As shown in Table 2, only BPA and DCF were found above the LOQ in bile samples. BPA were frequently found ($> 80\%$) with concentrations up to 0.89 $ng \cdot mL^{-1}$, while DCF were only detected in $< 20\%$ with concentrations up to 3.38 $ng \cdot mL^{-1}$. Fish bile has been used to detect exposure and uptake of different compounds in fish, such as estrogenic contaminants and polycyclic aromatic hydrocarbon (PAH) [17,18]. Kallio et al. [19] and Mehinto et al. [20] studied the uptake of diclofenac by exposing rainbow trout (*Oncorhynchus mykiss*) to water at environmentally relevant concentrations and found that diclofenac accumulated in the bile by a factor of between 320 to 950.

Concentrations of PPCPs and EDCs detected in fish blood are given in Table 2. Of the seven chemicals that were detected, GFB was the most abundant compound, with mean concentration of 8.98 $ng \cdot mL^{-1}$, followed by TCS and DCF, with mean concentrations of 7.30 and 5.33 $ng \cdot mL^{-1}$, respectively.

Four compounds were detected in muscle samples at concentrations exceeding LOQs. ESR, NPX and TCS were occasionally detected in $< 30\%$. BPA was found in all the samples with mean concentrations of 7.26 $ng \cdot g^{-1}$. These results are comparable to Ramirez et al. [21], where they found PPCPs at levels up to 19 $ng \cdot g^{-1}$ in fillet of fish in U. S. rivers.

3.4 Environmental implications

PCPPs and EDCs have been detected in water bodies around the world. In this study, some PCPPs and EDCs can accumulate to part per billion ($ng \cdot mL^{-1}$ or $ng \cdot g^{-1}$) levels in fish and were at least two orders of magnitude higher than the concentrations in lake water. Do the potential dietary intakes would present certain degrees of harm to human and/or ecological health over the long run? It remains an open question [1]. Base on the representativeness of analyzed samples, we selected the most abundant chemical in fish muscle, BPA, as target compound to assess its potential risk to human health.

BPA is an industrial chemical used in the manufacture of polycarbonate plastics and epoxy resins, such as baby feeding bottles, epoxy food-can linings, and other consumer plastics, with annual production of over three billion kilograms worldwide [22]. BPA was detected in most of the canned food, soft drink, infant formulas samples in North America [23–26]. BPA has also been detected in human breast milk and urine, with levels up to

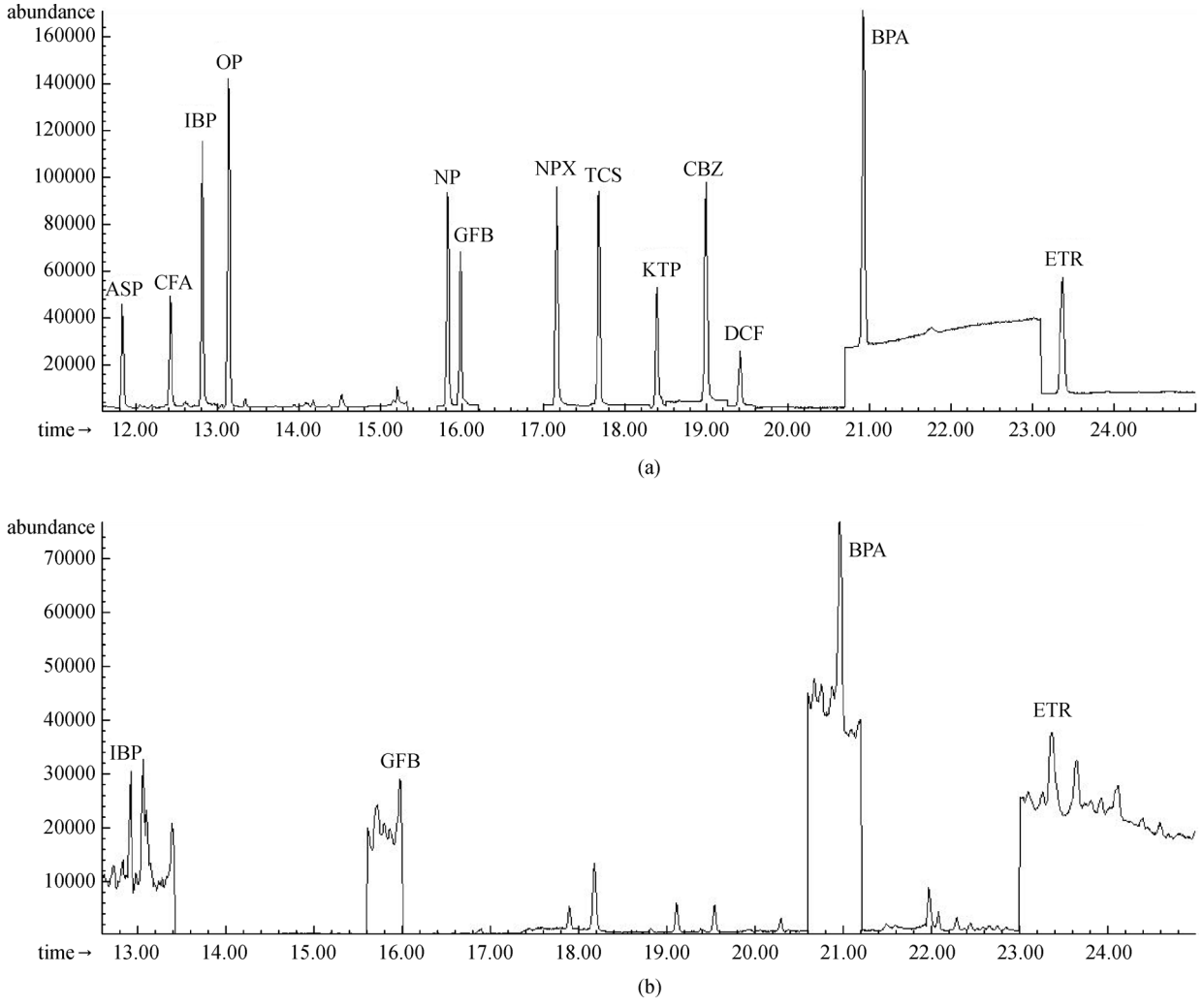

Fig. 1 GC-MS-SIM chromatograms of analytes: (a) standard solution of 13 analytes, 50 ng·mL^{-1}; (b) fish blood sample

Table 1 Analytical method performance

	recovery/(mean±SD, %)			limits of quantitation (LOQ)		
	bile	blood	muscle	bile/(ng·mL^{-1})	blood/(ng·mL^{-1})	muscle/(ng·g^{-1})
aspirin (ASP)	60±2.8	65±2.3	63±3.4	1.33	1.23	1.27
bisphenol A (BPA)	92±2.2	90±2.8	84±3.1	0.22	0.22	0.24
carbamazepine (CBZ)	68±4.5	72±4.4	62±4.8	0.59	0.56	0.65
clofibric acid (CFA)	83±6.7	76±4.6	82±6.4	0.96	1.05	0.98
diclofenac (DCF)	85±6.2	79±5.7	78±6.6	2.35	2.53	2.56
estrone (ETR)	90±6.8	95±4.8	80±5.3	0.89	0.84	1.00
gemfibrozil (GFB)	62±4.7	68±4.5	71±6.3	1.29	1.18	1.13
ibuprofen (IBP)	80±4.4	81±3.2	85±5.0	0.50	0.49	0.47
ketoprofen (KTP)	86±5.2	82±3.6	74±5.8	0.93	0.98	1.08
4-*n*-nonylphenol (NP)	65±3.8	70±4.3	72±5.3	0.62	0.57	0.56
naproxen (NPX)	78±6.5	76±3.8	91±6.8	0.51	0.53	0.44
4-tert-octylphenol (OP)	63±4.6	67±4.0	69±5.5	0.32	0.30	0.29
triclosan (TCS)	73±5.7	75±5.9	86±6.9	0.55	0.53	0.47

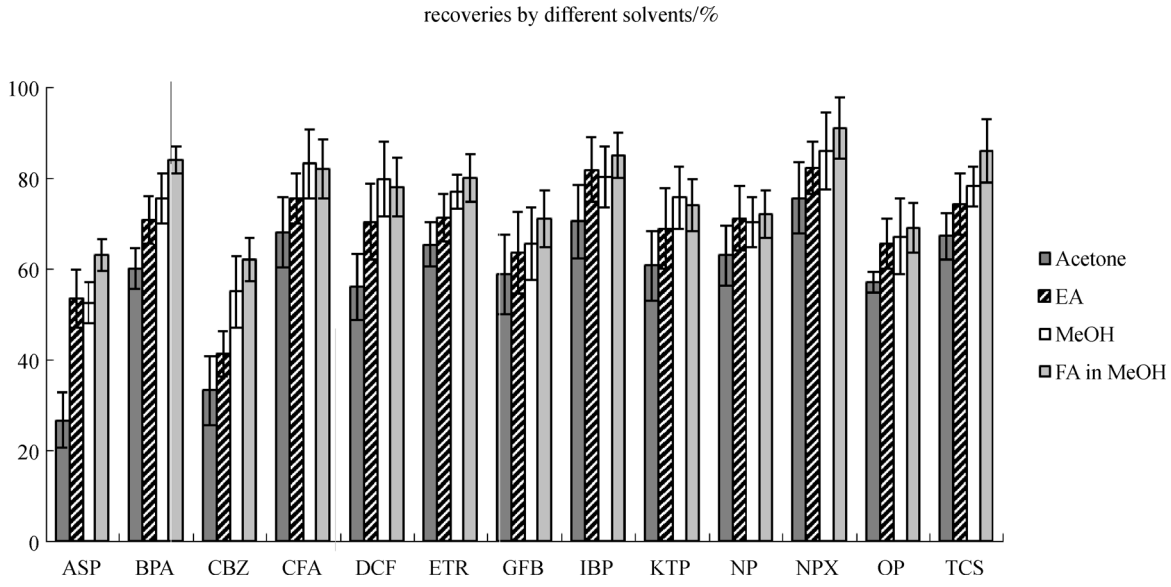

Fig. 2 Recovery and precision of the method optimized by different solvents. EA: ethyl acetate; FA in MeOH: 1% formic acid in methanol

Table 2 Concentrations of 13 compounds in fish

	lake water/ ($ng \cdot L^{-1}$)	bile/($ng \cdot mL^{-1}$)				blood/($ng \cdot mL^{-1}$)				muscle/($ng \cdot g^{-1}$)			
		min	max	mean	median	min	max	mean	median	min	max	mean	median
ASP	0.68	–	–	–	–	–	–	–	–	–	–	–	–
BPA	43.9	–	0.89	0.61	0.54	1.18	6.21	3.06	2.63	5.06	8.94	7.26	7.40
CBZ	0.35	–	–	–	–	–	–	–	–	–	–	–	–
CFA	–	–	–	–	–	–	–	–	–	–	–	–	–
DCF	5.6	–	3.38	2.97	–	2.88	10.5	5.33	4.4	–	–	–	–
ETR	1.1	–	–	–	–	2.98	7.52	4.63	3.57	–	1.18	1.09	–
GFB	1.7	–	–	–	–	5.34	13.1	8.98	8.74	–	–	–	–
IBP	1.3	–	–	–	–	1.22	5.07	3.09	2.62	–	–	–	–
KTP	–	–	–	–	–	–	–	–	–	–	–	–	–
NP	0.15	–	–	–	–	–	–	–	–	–	–	–	–
NPX	19.4	–	–	–	–	1.04	2.11	1.56	1.46	–	1.29	0.97	
OP	0.5	–	–	–	–	–	–	–	–	–	–	–	–
TCS	2.2	–	–	–	–	5.58	10.1	7.30	6.84	–	0.74	0.59	–

Note: –: < LOQ

6.3 and 30.1 $ng \cdot mL^{-1}$, respectively [27,28].

The primary route for human exposure to BPA is via contaminated foods and beverages [29]. We calculated Estimated Daily Intakes (EDI) of BPA by fish consumption for U.S. resident with maximum. Annual per capita consumption of fish (boneless, trimmed weight) is 7.3 kg per year [30]. Average body mass in North America was 80.7 kg [31]. Therefore, the EDI of BPA, on a body weight (bw) basis, is 2.2 $ng \cdot kg^{-1}$ $bw \cdot d^{-1}$.

To assess potential public health risks, hazard index (HI) was calculated by dividing the EDI of BPA by the Tolerable Daily Intake (TDI). The HI value must be lower than unity to ensure the intake dose below TDI, and thus an acceptable risk to human. European Food Safety Authority (EFSA) [32] decided that the TDI of BPA was determined to be 50 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$, which is the amount of BPA a consumer can safely ingest without harm over a whole lifetime. The calculated HI (4.4×10^{-5}) was far less than unity and suggested that the adverse health effects of BPA associated with fish diet is minimal. However, given the possibility of ingesting multiple foods, drinks and other sources of exposure to BPA, it is imperative to continue investigating the BPA levels in U.S. food, water supply, and other potential pathways of exposure to BPA.

4 Conclusions

A method for the simultaneous determination of 13 EDCs and PPCPs by GC-MS in fish was developed. The methods were applied to the analysis of fish samples from the Lake Skinner, California. Seven chemicals were detected at part per billion levels in fish, i.e., $ng \cdot mL^{-1}$ in bile and blood, and $ng \cdot g^{-1}$ in muscle. EDI of BPA through fish diet was 179 ng $\cdot d^{-1}$ on average for U.S. resident. Risk assessment showed that the exposure to BPA from fish diet is unlikely to pose a health risk. However, it is important to monitor the BPA levels in other exposure routes and its risk to human health. Further studies should also focus on the occurrence, behavior and fate of PPCPs and EDCs in environment.

Acknowledgements We thank Frederick Ernst for their support during the sample collection.

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s11783-014-0640-6 and is accessible for authorized users.

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