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Butyltin Compounds in Fish Commonly Sold in North of Iran

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Abstract Levels of tributyltin (TBT) and its degradation products, mono (MBT) and dibutyltin (DBT), were monitored in fish commonly consumed in Iran. Samples were purchased from fish markets at seasonal intervals in 2010 along the North coast of Iran. Concentration of MBT, DBT and TBT in the muscle of these fish were in the range of n.d. -1.01 ± 0.84, n.d. -0.33 ± 0.26 and n.d. -4.31 ± 0.95 ngg^{-1} (wet weight), respectively. Residue levels of butyltins were found lower than the tolerable average residue level of 100 ngg^{-1} (wet weight).

Keywords Organotin compounds · Fish · Iran

Organotin compounds are considered to be the endocrine-disrupting chemicals (Horiguchi et al. 1998). Environmental concern about organotin compounds (OTC) has considerably increased owing to uncontrolled use of tributyltin (TBT) in antifouling paints and triphenyltin (TPhT) in pesticides in the past (Morabito et al. 1995). As a result of the long-term application of TBT-based paints, relatively high levels can be found in harbors, waterways and shipping lanes (Morabito et al. 1995). Highly sorbet on suspended matter and sediment, TBT can still be encountered in the marine environment although its use in paints has been restricted for almost a decade (Maguire 2000) The direct results of the use are that considerable amounts of organotin compounds have entered marine environments and their residues have subsequently been found in many organisms, including algae, mollusks, fish (Oh 2009; Yang et al. 2008), seabirds (Guruge et al. 1997) and marine mammals (Tanabe 1999). Several investigations have shown that seafood is the primary source of human exposure to organotin compounds either in Asia or Europe (Toyoda et al. 2000; Rantakokko et al. 2006). Through the consumption of contaminated seafood, organotin compounds may enter the human body. In Iran the use of organotin compounds in antifouling agents was restricted to boats under 25 m in length in 2003. However, butyltin compounds (BTs) were still encountered in water and sediments (Liu et al. 2006). This was attributed to TBT leached from antifouling paints from big commercial vessels (larger then 25 m in length) and/or the persistence of TBT in sediments (Dowson et al. 1996). The Caspian Sea is the largest enclosed body of water in the world with a surface area of about 436,000 km^2. The coastlines of the Caspian Sea are shared by Iran, Azerbaijan, Kazakhstan, Russia and Turkmenistan (Zeynali et al. 2009). Referring to scientific literature, very few data have been published about the accumulation of toxic elements in fish of the Caspian Sea. Additionally, fish has an important role as a source of protein in the diet of people in northern states of Iran. Therefore, this study was aimed to determine the concentrations of butyltin compounds mono (MBT), di (DBT) and tributyltin (TBT) in edible muscle of frequently consumed fish species, collected from different fishing sites of Iranian coastal waters of the Caspian Sea in two separate seasons.

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Materials and Methods

Between March 2010 and November 2010, five most dietary and economically important fish species, namely Caspian white fish (Rutilus frisii kutum), Golden gray mullet (Liza aurata), Caspian salmon (Salmo trutta caspius), Pike (Esox lucius) and Common carp (Cyprinus carpio), were purchased from the fish markets from four fishing sites (Chaloos, Anzali, Roodsar and Fereidonkenar) of Iranian coastal waters of the Caspian Sea at seasonal intervals. Five different samples of each species were obtained from each site. The samples were brought to the laboratory on ice inside an ice chest. Then, the muscle samples were taken, packed in polyethylene pouches and stored at −20°C prior to analysis. Butyltin compounds were analyzed using head-space solid phase microextraction coupled to gas chromatography equipped with single quadrupole mass detection (GC–MS). To about 1 g of homogenized muscle samples, 100 µl of tripropyltin chloride (TPT; 50 ng mL^{-1}) as an internal standard and 15 mL of aquatic tetramethylammonium hydroxide (25% w/v) were added. To optimize the dissolution, samples were stirred in an ultrasonic bath for 1.5 h at 50°C and centrifuged at 3,000 rpm for 10 min then. For HS-SPME sampling, 10 mL of the supernatant, and 2 mL of acetate buffer solution (pH 5.3) were placed in a 20 mL glass vial. 200µL of 2% (w/v) $NaBEt_4$ was added and the vial was then immediately closed with a PTFE-coated silicon rubber septum. The Single-walled carbon nanotube coated fiber which was developed in the previous study (Rastkari et al. 2010), was inserted into the bottle, and situated at about 1 cm above the surface of the aqueous phase. The bottle was moved to a 45°C water bath, where the solution was mixed for 30 min with a glassy magnetic stirring bar, allowing in situ derivatization and extraction to the fiber. After the extraction, the fiber was inserted into the GC injector for thermal desorption. The SPME holder was adjusted so that the exposed fiber tip was positioned about halfway (3.8 cm) into the GC injection port when extended from the protective needle. Injection was accomplished by extending the fiber in the heated inlet for 4.5 min, and the splitter was opened after 3 min. The additional 1.5 min of exposure time in the injection port allowed the fiber to be cleaned of any compound that was not desorbed in the initial 3 min. Preliminary studies indicated that the above procedure allowed for reproducible, quantitative transfer of MBT, DBT and TBT into the injector of the GC system. Blank samples containing internal standard were analyzed at the beginning and at the end of the samples queue. Each sample was extracted in triplicate and the average response was considered for quantification. The instrument used for GC–MS analysis was an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890 plus gas chromatograph equipped with a 5,973 mass selective detector quadrupole mass spectrometer. The gas chromatograph was fitted with an HP-5MS column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The instrumental temperatures were as follows: injector temperature, 260°C; initial oven temperature, 40°C (held for 1 min), increased to 160°C at a rate of 30°C min^{-1}, then increased to the final temperature 260°C at a rate of 20°C min^{-1}, where it was held for 2 min. The inlet was operated in splitless mode. The temperature of the transfer line was maintained at 270°C. Helium (99.99%) was used as carrier gas at 1.2 mL min^{-1} (constant flow). The source and quadrupole temperatures were kept at 230 and 150°C, respectively. The electronic beam energy of the mass spectrometer was set at 70 eV. The mass selective detector was operated in electron impact (EI) mode using selected ion monitoring (SIM). The dwell time of each ion was set at 100 ms. The GC conditions were selected to minimize the time of analysis while allowing all the analytes to elute in acquisition groups containing suitable number of ions for monitoring (Table 1). The LOQ values for MBT, DBT and TBT were 0.01, 0.03, 0.05 ngg^{-1}. Mean concentrations of MBT, DBT and TBT among five groups of selected fish were analyzed and because the distribution of data might not be normal; the analysis was carried out by means of two statistical procedures: analysis of variance (one way ANOVA) and Kruskal–Wallis test. Results were expressed as mean ± SD and 95% confidence intervals. The level of significance was set to 0.05 and p values > 0.05 were assumed to be non significant.

Results and Discussion

Without taken into account the fishing sites, the average concentrations of butyltin compounds in fish species caught from Iranian coastal waters of the Caspian Sea are shown in Table 2. Concentrations of MBT, DBT and TBT in the muscle were in the range from n.d. to 1.01 ± 0.84 ngg^{-1}, n.d. to 0.33 ± 0.26 ngg^{-1} and n.d. to 4.31 ± 0.95 ngg^{-1} (wet weight), respectively (as shown in Table 2). The highest concentrations of MBT, DBT and TBT were found in the muscle of Caspian salmon and the lowest at Caspian

Table 1 Selected ions used for the detection of target analytes by GC–MS in SIM mode

Ion group	Analyte	Formula structure of derivative	Molecular ion (m/z)	Selected ion (m/z)	Confirmed ions (m/z)
1	MBT	Sn^+ $BuEt_3$	264	235	233,207
1	TPT	Sn^+ Pr_3Et	278	249	247,221
2	DBT	Sn^+ Bu_2Et_2	292	263	261,235
2	TBT	Sn^+ Bu_3Et	320	291	289,263

Table 2 Mean concentration (SD) of butyltin compounds (ngg^{-1}, wet weight) in fish samples

Date	Fish	Sample size (n)	MBT	DBT	TBT
March 2010	Caspian white fish	12	n.d.	n.d.	n.d.
	Golden gray mullet	12	n.d.	n.d.	n.d.
	Caspian salmon	12	0.4 (0.14)	0.22 (0.19)	1.26 (0.24)
	Pike	12	n.d.	n.d.	n.d.
	Common carp	12	0.10 (0.09)	n.d.	0.21 (0.12)
June 2010	Caspian white fish	12	0.12 (0.16)	n.d.	0.38 (0.16)
	Golden gray mullet	12	0.11 (0.13)	n.d.	0.65 (0.32)
	Caspian salmon	12	1.01 (0.84)	0.19 (0.11)	4.31 (0.95)
	Pike	12	0.31 (0.19)	0.11 (0.08)	0.85 (0.21)
	Common carp	12	0.16 (0.11)	n.d.	0.46 (0.24)
September 2010	Caspian white fish	12	n.d.	n.d.	0.20 (0.14)
	Golden gray mullet	12	0.10 (0.11)	n.d.	0.67 (0.35)
	Caspian salmon	12	0.88 (0.27)	0.33 (0.26)	4.11 (2.25)
	Pike	12	0.28 (0.11)	0.09 (0.06)	0.65 (0.31)
	Common carp	12	0.12 (0.09)	n.d.	0.41 (0.26)
November 2010	Caspian white fish	12	n.d.	n.d.	0.09 (0.05)
	Golden gray mullet	12	n.d.	n.d.	0.11 (0.09)
	Caspian salmon	12	0.48 (0.21)	n.d.	1.36 (0.58)
	Pike	12	0.08 (0.06)	n.d.	0.14 (0.11)
	Common carp	12	0.07 (0.04)	n.d.	0.23 (.012)
P value*		–	–	<0.001	<0.001
P value**		–	–	<0.001	<0.001

n.d. not detected

* one-way ANOVA

** Kruskal–Wallis test

white fish. Although the concentration of each butyltin compound (MBT, DBT and TBT) varied greatly for different individuals, different species, and different seasons, TBT was prevalent in most of the muscle samples determined. Concentrations of MBT and TBT in the muscle of all fish were significantly different ($p < 0.05$, by ANOVA test) from different months, but concentrations of DBT, only in the muscle of Caspian salmon and Pike were significantly different ($p < 0.05$, by ANOVA test) from different months. We also found that the mean concentrations of MBT and TBT were higher in fish caught in June than in the other months, but there was no clear seasonal trend for DBT. Different from our results, Dong et al. (2004) found that ponyfish Leiogenathus splendens collected from the west coast of Taiwan contained higher BTs in winter than in other seasons. In Italy, 4 years after the restrictions on the usage of organotins, DBT and TBT were detected in the range of 1–26 and 12–260 ng/g wet weight, respectively (Amodio-Cocchieri et al. 2000). Six years after the ban on the use of TBT for all coastwise vessels and aquaculture facilities was enacted in Japan, the TBT concentration in the muscle of 11 species of fish from the port of Osaka and Yodo River was detected to be in the range of 11–182 ng/g wet weight (Harino et al. 2000). In the France, the TBT concentration in fish collected from the market during 2005 ranged between 1.1 and 23 ng/g wet weight (Guérin et al. 2007). These results together with recent reports on the butyltin pollution in seawater and sediments (Strand et al. 2003; Murai et al. 2005) have shown that the sources of TBT contamination still remain worldwide, and that the partial ban on TBT usage was not sufficient to reduce the threat of butyltin compounds to human health. Seafood consumption is the main source of human dietary exposure to butyltin compounds. The toxicology of butyltin compounds in humans has not yet been fully resolved (WHO 1990). On the basis of TBT's ability to reduce the immune function (Penninks 1993) FAO suggested a tolerable daily intake (TDI) for TBT of 250 ng/kg body weight/day. Various attempts have thus been made to determine whether the level of organotin intake by humans from eating marine food should be a cause for concern. The tolerable average residue level (TARL) is defined as the level in seafood that is tolerable for the average consumer with an average body weight of 60 kg. TARL = (TDI × 60 kg body weight)/Average daily seafood consumption. In Iran an average 150 g/day/person of seafood was consumed.

Using this value, an estimated 100 ng/g (wet weight) of TARL for TBT in seafood was obtained. Heidrich et al. (2001) also reported that human placental aromatase activity is directly inhibited by TBT (IC50 = 6.2 μM) or DBT but not by MBT and tetrabutyltin. Therefore, concentrations of DBT plus TBT in the muscle or the internal organ were taken while considering the residue level of butyltins. TBT were detectable in the most of our samples. The highest concentrations of DBT plus TBT were found in Caspian salmon (0.19 ± 0.11 and 4.31 ± 0.95 ng/g, respectively) purchased in June. Considering the dry/wet ratio of fish muscle, TBT concentrations in seafood caught from the North coast of Iran were lower than those from other markets in the world. Concentration of DBT plus TBT in muscle of all these tested fish did not exceed the TARL value of 100 ng/g (wet weight).

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