ANTHROPOGENIC AND NATURALLY-PRODUCED ORGANOBROMINATED COMPOUNDS IN BLUEFIN TUNA FROM THE MEDITERRANEAN SEA

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Abstract

Anthropogenic compounds, such as polybrominated diphenyl ethers (PBDEs), together with naturally-produced organobromines, such as methoxylated PBDEs (MeO-PBDEs), polybrominated hexahydroxanthene derivates (PBHDs), 2,4,6-tribromoanisole (TBA) and a mixed halogenated monoterpene (MHC-1), were measured in muscle from 26 farmed and wild bluefin tuna (*Thunnus thynnus*) caught in the Mediterranean Sea. This species is ecological attractive because of the changes of geographic habitat throughout its long lifespan which affect its feeding and because it is a part of human diet (especially for people living on coastal areas). PBDE concentrations were similar between tuna samples of different groups (17-149 ng/g lw in farmed tuna, 25-219 ng/g lw in longline-fished tuna and 26-126 ng/g lw in net-fished tuna). However, higher concentrations of naturally-produced MeO-PBDEs and PBHDs were observed in the two types of wild tuna (longline-fished and net-fished) compared to farmed tuna suggesting that wild tunas come easily in contact with sources of these compounds. In all cases, PBHDs presented the highest contribution to the sum of organobromines (50% in farmed tuna and > 90% in wild tuna). TBA was detected at low concentrations (< 6 ng/g lw), while MHC-1 was found at higher levels (up to 42 ng/g lw) in farmed tuna. The estimated daily ingestion of PBDEs from tuna was 830 ng PBDEs/day, regardless of the origin of the tuna. While this value is approximately 600 times lower that the minimum risk level set by the U.S. Department of Health and Human Services, it is approximately 8 times higher than the total intake of PBDEs via diet, suggesting that consumption of tuna can add considerably to the total daily intake of PBDEs.

Introduction

Brominated flame retardants (BRFs), such as polybrominated diphenyl ethers (PBDEs), have been increasingly investigated during the last decade because of their potential for bioaccumulation and contamination in the environment and food webs and because of their potential health effects[1,2]. PBDEs are synthetic compounds used in a large variety of products such as plastics, textiles and electrical equipment and their use has been gradually restricted by the European Union since 2004[3]. More than 4000 halogenated naturally-produced compounds have also been found in the marine environment, while only a few of them, such as -the structural PBDE analogues- methoxylated PBDEs (MeO-PBDEs), polybrominated hexahydroxanthene derivates (PBHDs), 2,4,6-tribromoanisole (TBA) and a mixed halogenated compound (MHC-1), have been measured at high concentrations in marine organisms, including top-predators[4]. Yet, only a few papers have reported on concentrations of PBDEs and MeO-PBDEs in Mediterranean Sea organisms so far**.**

The present study focuses on bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea, an economically and gastronomically relevant species. Tuna populations present seasonal variations, the Mediterranean area being massively populated during warmer seasons. Remarkably, populations have considerably decreased in the last decades (almost by 80%) most likely due to over-fishing by industrial fisheries. The bluefin tuna is a flexible species, capable of adapting to its environment which changes rapidly due to their temporal migration. Moreover, this species is also suitable for aquaculture. They feed on crustaceans, small fishes and cephalopods in their juveniles stages, and rely on large cephalopods and pelagic fishes as adults. Because of all these confounding factors, these animals may exhibit different bioaccumulation profiles, which make this species interesting from an ecotoxicological point of view.

This paper aims at assessing the presence of anthropogenic and naturally-produced organobrominated compounds in bluefin tuna from the Mediterranean Sea. Differences in concentrations and accumulation profiles of these brominated compounds between wild and farmed tuna were investigated. Moreover, the daily human intake of brominated compounds was estimated from the ingestion of Mediterranean tuna and its contribution to the total diet evaluated.

Materials and methods

Sample collection. Muscle samples from 26 bluefin tuna (12 males and 14 females between 2-13 yrs old) were collected in the Mediterranean Sea (South Thyrrenian Sea) during 2003 by different fishing techniques. Twenty wild animals were used from which 10 were caught by longline fishing (baited hooks hanging from a single line) and 10 by "mattanza" technique (net chambers). Six tuna originated from a fish farming area. Fishes were dissected and muscle samples were frozen at -20 ºC until analysis.

Chemicals and materials. All solvents used for the analysis were of SupraSolv grade (Merck, Darmstadt, Germany). Anhydrous sodium sulfate and silica gel (Merck) were washed with *n*-hexane and used after activation by heating overnight at 160 ºC. Empty polypropylene columns for clean-up (25 mL) were purchased from Alltech (Lokeren, Belgium). Nine PBDE congeners, fifteen MeO-BDEs, two PBHDs isomers, MHC-1 and TBA were targeted in the samples (see Table 1). BDE 77 and 1,2,3,4-tetrachloronaphtalene (1,2,3,4-TCN) were used as internal standard and syringe standard, respectively. Standards of PBDE and MeO-PBDE congeners were purchased from Wellington Laboratories (Guelph, ON, Canada) and from Accustandard (New Haven, CT, USA). TBA was purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). A mixture of triBHD and tetraBHD at 1.6 ng/µL in isooctane and a standard of MHC-1 at 1.9 ng/µL also in iso-octane were a gift from Dr. Walter Vetter (University of Hohenheim, Germany).

Sample preparation. The analytical procedure for determination of organobrominated compounds has been previously described[5]. Briefly, typically 3 g of tuna muscle were accurately weighed and grounded with sodium sulfate. The mixture was spiked with 4 ng of BDE 77 and extracted for 2 h in a hot Soxhlet (Büchi, Flawil, Switzerland) with 100 mL of *n*-hexane:acetone (3:1; v/v). The extract was evaporated and cleaned-up on acid silica (~8 g) using 20 mL *n*-hexane and then 15 mL of dichlorometane as elution solvents. The extract was concentrated and further evaporated under a nitrogen stream to near dryness and redissolved in 100 µL of recovery standard TCN (used to calculate the recovery of the internal standard). The lipid content was determined gravimetrically on an aliquot of the extract by solvent evaporation in an oven (105 ºC, 1 h).

Analysis. For the determination of target analytes, each extract was injected in two systems. An Agilent 6890-5973 GC-MS system operating in the electron ionization (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperature were set at 230, 150 and 300 ºC, respectively. One µL of the cleaned extract was injected in cold pulsed splitless mode. Helium was used as carrier gas at constant flow (1 mL/min). The mass spectrometer was used in selected ion monitoring (SIM) mode and the two most abundant ions from the cluster were monitored for each homologue group of compounds with a dwell time of 40 ms.

All extracts were injected also in an Agilent 6890-5973 GC-MS system operated in electron capture negative ionization (ECNI) mode and equipped with a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperature were set at 250, 150 and 300 ºC, respectively. Helium gas was used as carrier gas at constant flow (1 mL/min). Methane was used as reacting gas. The electron multiplier voltage was set at 2200 V. One µL of the cleaned extract was injected in solvent vent mode. The mass spectrometer was operated in SIM mode by monitoring bromine isotope ions (*m/z* 81 and 79) with a dwell time of 50 ms. Additional details can be found in a previous report[5].

Quality assurance and quality control. Multi-level calibration curves in the linear response interval of the detector ($r^2 > 0.99$) were created for the quantification. The identification of each analyte was based on the simultaneous detection and ratio between the corresponding ions and on the relative retention times (RRTs) to the internal standard. For most compounds, results obtained by EI were used since concentrations were high

enough and selectivity was improved through the monitoring of specific ions from the molecular cluster. Yet, for some compounds (e.g., BDE 28), results obtained by ECNI were used since co-elutions were not observed here. Nevertheless, results obtained for BDE 47 by EI and ECNI did not differ by more than 10% from each other. The quality control was also performed through analysis of procedural blanks, a replicate sample and a standard reference material (SRM 1945 whale blubber which has certified values for PBDEs). For the replicate and SRM 1945, the relative standard deviations (RSDs) were < 10% with few exceptions at very low concentrations**.** Recoveries of the IS were between 96-120% (mean, 106%; RSD < 8%). PBDEs, MeO-PBDEs, TBA, PBHDs and MHC-1 were not detected in the procedure blanks in the EI mode analysis. Nevertheless, BDE 47, BDE 100 and BDE 99, 2-MeO-BDE 68 and TBA were detected in ECNI mode and the mean value of each analyte in the procedural blanks was used for subtraction. Method limits of quantification (LOQ) were set at 3×SD obtained in the procedural blanks. LOQs ranged from 0.1 to 0.25 ng/g lw for individual PBDE and MeO-PBDE congeners, and were 1 ng/g lw for the two PBHD isomers and 0.1 ng/g lw for TBA and MHC-1.

Statistical analysis. For calculations of sums and means**,** congeners with concentrations below LOQ were replaced by a value of ½ × LOQ. Differences between concentrations of organobromine compounds in the three fish groups (longline, "mattanza", and farmed) were investigated by one-way ANOVA. Grubb´s test was employed to detect outliers between samples caught by the same fishing technique. Limit of statistical significance was set at 0.05.

Results and Discussion

Lipid content: Lipid percentages differed according to the origin of the tuna samples (Table 1). Farmed tunas presented higher values probably due to a combination of an intensive diet (mostly based on herring) and a lower activity compared to their wild counterparts. A narrow range (between 27 and 37%, mean 32%, RSD < 12%) was observed for farmed tuna due to the identical conditions in which they were kept**.** A wider lipid content range was found in wild fishes with percentages ranging from 2.7 to 30%, but independent from tuna age, length and gender (ANOVA, all $p > 0.05$).

PBDEs: The investigated PBDE congeners were detected in all three fish groups: **farmed**, longline fished **(wild 1)** and net-fished tunas **(wild 2)**. The PBDE profiles were similar in all three cases with BDE 47 being the predominant congener (mean 44%, RSD < 3%), followed by BDE 100 (mean 19%, RSD < 4%) and BDE 99 and 154 (mean 9%, RSD < 5%)**.** Total PBDE concentrations (sum of PBDEs) varied largely between samples of the same group (17-149 ng/g lw in farmed tuna, 25-219 ng/g lw in 'wild 1' tuna and 26-126 ng/g lw in 'wild 2' tuna) (Table 1). However, no statistical significant differences were found between PBDE levels in the three tuna groups (ANOVA, all $p > 0.05$). No associations were found between morphologic characteristics, i.e. gender, age and length, and the total content of PBDEs.

In similar studies, identical PBDE profiles were found with BDE 47 being the most predominant congener (> 50 % of total PBDEs), and other PBDEs varying in the order BDE 99 ≥ BDE 100 ≥ BDE 154. However, median concentrations found in different marine organisms ranged largely from lower levels found in fishes from open areas worldwide[6] to higher levels measured in marine mammals from the Mediterranean Sea[7].

MeO-PBDEs: Only 8 out of the targeted 15 MeO-PBDE congeners were found at measurable levels in the studied tuna samples (Table 1). MeO-PBDE profiles were similar in the three investigated groups, with 2´-MeO-BDE 68 and 6-MeO-BDE 47 contributing with almost 98% of the sum MeO-PBDEs, which agrees with reported values from the literature for marine species[8]. A shift in the ratio 2´-MeO-BDE 68 to 6-MeO-BDE 47 was observed between wild and farmed tunas indicating sponges as the dominant source of MeO-PBDEs. A higher proportion of 6-MeO-BDE 47 would point to algae as the principal source of MeO-PBDEs[9]. Our results support this hypothesis mostly explained by the restriction in mobility of farmed tuna and, as a consequence, a reduced direct contact with sponges. Concentrations of MeO-PBDEs in tuna samples were in the same range as PBDE levels and varied from 47 to 74 ng/g lw in farmed tuna, from 73 to 248 ng/g lw in wild 1 tuna, and from 69 to 503 ng/g lw in wild 2 tuna (Table 1). Significant differences between MeO-PBDE concentrations were found between farmed and two wild groups of tuna (ANOVA, $p< 0.01$).

The literature on MeO-PBDEs in fish from different locations is rather scarce and only a few reports of these compounds suggest that, while MeO-PBDE congeners were not detected in lower organisms and sediments, they can biomagnify in aquatic food chains, reaching higher concentrations in top-predators[10].

PBHDs: PBHDs have recently been reported as naturally-produced brominated compounds originating from sponges[4]. PBHD isomers detected in this study showed the highest concentrations among all brominated compounds measured representing around 50% of the sum of organobromine compounds in farmed tuna, and more than 90% of the total content in wild tuna (see Figure 1), probably due to the higher interaction of these animals with natural sources (e.g., sponges). PBHD concentrations differed largely between farmed and wild tunas, with the concentrations in the wild tuna being one order of magnitude higher than those in farmed tuna (Table 1).

Table 1. Concentrations of organobrominated compounds (ng/g lipid weight) in muscle of bluefin tuna (*Thunnus thynnus)* from the Mediterranean Sea.

	Farmed tuna (n=6)		**Wild 1 (n=10)**		**Wild 2 (n=10)**	
	Median	**Range**	**Median**	**Range**	**Median**	**Range**
Lipids (%)	32	27-37	17	3.2-30	7	2.7-27
BDE 28	1.2	0.4-5.3	1.1	0.5-2.4	0.8	0.5-2.2
BDE 49	2.1	0.9-8.8	2.6	1.4-8.1	2.6	1.9-7.0
BDE 47	16	7.7-75	26	11-109	23	11-53
BDE 66	3.3	1.3-6.8	4.0	1.3-20	2.7	<0.1-9.9
BDE 100	6.0	3.0-30	11	4.3-40	9.4	4.5-22
BDE 99	3.1	0.8-7	3.7	2.8-13	6.5	2.3-17
BDE 155	1.3	0.7-5	1.9	0.7-5.4	1.4	<0.2-3.7
BDE 154	3.0	1.6-11	4.9	1.9-14	5.1	1.7-11
BDE 153	1.0	0.3-2	1.1	0.5-2.5	1.0	<0.2-2.1
Sum PBDEs	**37**	**17-149**	**58**	**25-219**	**53**	**26-126**
6-MeO-BDE 49	0.6	0.5-1	1.0	0.5-1.4	1.0	<0.1-2.8
2´-MeO-BDE 68	16	8.2-20	45	23-80	53	25-250
6-MeO-BDE 47	50	38-54	57	37-163	74	44-246
5- MeO-BDE 47 + 4- MeO-BDE 49	0.1	<0.1	0.3	<0.25-0.4	0.2	<0.1-1.1
2-MeO-BDE 28	0.3	0.2-0.4	0.4	<0.25-1.1	0.4	<0.1-0.6
4-MeO-BDE 17	0.1	<0.1-0.2	0.1	<0.25-0.5	0.1	<0.1
6-MeO-BDE 99	0.1	<0.1	0.2	<0.25-0.9	0.1	<0.1
Sum MeO-PBDEs	**68**	**47-72**	**110**	**73-248**	**134**	**69-503**
Tri-BHD	43	37-73	1229	117-2199	1652	854-4443
Tetra-BHD	58	40-578	1892	183-6187	2391	1713-11165
Sum PBHDs	**101**	**78-651**	**3157**	**300-8269**	**4029**	**2678-15606**
TBA	0.8	0.4-1.0	3.5	0.8-5.2	4.1	2.0-6.4
MHC-1	49	<0.1-54	24	<0.1-47	27	15-66

[a]For congeners with concentrations below LOQ, the mean concentrations were calculated using a value of ½ × LOQ.

PBHDs have been found in the Mediterranean sponge (*Scalarispongia scalaris)* and in different marine species, such as sea bass, sardines and anchovies[4], all of these fish constituting an important part of the diet of the bluefin tuna. In most of these samples, the PBHD profile was different than the one observed in tuna, with triBHD being

more abundant than tetraBHD. The abundance of tetraBHD could be an indication of either metabolic breakdown of triBHD in tuna, a higher bioaccumulation capacity for tetraBHD, or a particular diet habit. Tuna might bioaccumulate PBHDs from the environment in relation to the natural background levels in seawater, or they might biomagnify these naturally-produced brominated compounds through consumption of prey which contain PBHDs.

TBA and MHC-1. These two organobrominated compounds were detected in all investigated tuna samples. Concentrations of TBA, which is produced mostly by algae, in Mediterranean bluefin tunas ranged from levels found in farmed fishes (0.4-1.0 ng/g lw) to higher levels measured in wild tunas (2-6.4 ng/g lw). These results suggest that there is little contact between algae and tuna. Higher concentrations of TBA were found in other marine samples with lower levels in free-living species.

MHC-1 levels detected in farmed tuna were slightly lower than those of PBDEs and MeO-PBDEs (mean value, 42 ng/g lw). In contrast, mean concentrations found in longline and net-fished tunas were 26 and 30 ng/g lw, respectively, representing < 1% of the total sum of organobromines. Lower MHC-1 concentrations were measured in other Mediterranean fishes, such as sea bass and deep-sea fishes, which suggests that natural sources of TBA and MHC-1 are scarce in the Mediterranean Sea.

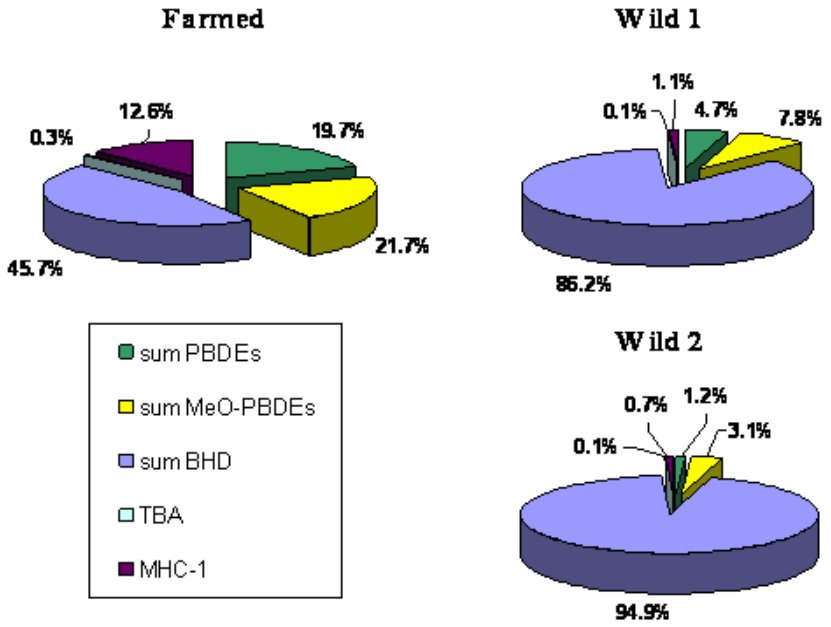

Figure 1 Organobrominated compound distribution in the three studied fish groups. (Wild 1 = longline fished tuna, Wild 2 = net-fished tuna).

Estimated daily ingestion (EDI): The significant contribution of fish and seafood to the dietary BFR intake in humans (~1/3 of the total), combined with the relevance of bluefin tuna in a suitable diet, emphasizes the necessity to control and monitor the presence of these pollutants in the diet. The minimum risk level (MRL) for PBDEs in humans has been set by the U.S. Department of Health and Human Services at 7 µg/kg body wt/day or 490 µg PBDEs/day for an adult of 70 kg.

By using the estimated daily fish consumption in Catalonia (Spain) set in 2006 at 68 g fish per day by a standard adult man (70 kg body weight), and assuming that tuna is the only fish eaten, the average EDI (considering the mean of sum PBDEs) was 12 ng/kg body wt/day (or 830 ng PBDEs/day). Mediterranean tuna is a common food item in the diet of Catalonians, but is not the only fish species eaten, so this value is probably an overestimation of reality. This result is approximately 600-fold lower that the MRL established by Agency for Toxic Substances

and Disease Registration (ATSDR). While there is no risk for human health the total PBDE ingested via tuna consumption is approximately 8 times higher than the total PBDE through diet[11], suggesting that consumption of tuna can add considerably to the total intake of PBDEs. However, the ATSDR limit does not take into account any other brominated compounds which can be present in much higher concentrations than PBDEs in wild tuna and for which no toxicological data are available. According to data from the present study for MeO-PBDEs and PBHDs, the average intake through tuna consumption is 2.2 and 34.8 µg/day, respectively.

In conclusion, anthropogenic and naturally-produced organobrominated compounds have been evidenced in Mediterranean tuna. Similar profiles were observed in farmed and wild tunas, but concentrations of naturally-produced compounds varied largely between these two animal groups. In agreement with previous reports from the Mediterranean Sea, PBHDs were the most abundant brominated compounds, suggesting that wild tunas may easily come in contact with possible sources, e.g. sponges. Results also prove that the estimated daily ingestion of PBDEs through tuna consumption do not pose a risk to human health. Yet, tuna contains many other toxic halogenated chemicals, which might show an unpredictable synergetic effect for consumers.

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