ANTHROPOGENIC AND NATURALLY-OCCURRING ORGANOBROMINES IN TWO DEEP-SEA FISH SPECIES FROM THE MEDITERRANEAN SEA

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Introduction

The presence of persistent organochlorine compounds, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), in deep-sea fish has been recently the object of several investigations[1-3]. These hydrophobic contaminants have been found in high concentrations in deep-sea fish, suggesting a vertical distribution of these contaminants throughout the water column[4,5], due to their association to organic particles, that finally reach the sea bottom. With the exception of a single study[3], newer contaminants, such as polybrominated diphenyl ethers (PBDEs) have not yet been investigated in deep-sea fish. Additionally, while naturally-occurring organohalogenated compounds (e.g. methoxylated PBDEs), have been reported in the marine environment[6-8], they have not been studied in deep-sea fish.

The present work focussed on two species of deep-sea fish from the Mediterranean Sea. On one hand, we investigated the concentrations and profiles of PBDEs contaminants in these fish species and, on the other hand, we also evaluated the presence of naturally-occuring organobrominated compounds (such as MeO-PBDEs).

Materials and methods

Samples: Liver samples originated from two species of deep-sea fish (the hollowsnout grenadier - *C. coelorhynchus* - abbreviated as CC, and the roughsnout grenadier - *T. trachyrinchus* - abbreviated as TT) caught in the Mediterranean Sea in 2006. Liver from fish samples with similar lengths were pooled to create a number of 6 (from 203 individuals) and 9 (from 307 individuals) pools for the CC and TT species, respectively.

Materials: Standards of PBDEs (IUPAC no. 28, 49, 47, 66, 85, 99, 100, 153, 154 and 183) and MeO-PBDEs (mix MEOBDES) were purchased from Wellington Laboratories, while additional MeO-PBDE standards were a gift from Accustandard. The following MeO-PBDE congeners were targeted: 2-MeO-BDE 7, 3-MeO-BDE 7, 2-MeO-BDE 28, 3-MeO-BDE 28, 4-MeO-BDE 17, 4-MeO-BDE 42, 3-MeO-BDE 47, 5-MeO-BDE 47, 6-MeO-BDE 47, 4-MeO-BDE 49, 2'-MeO-BDE 68, 4-MeO-BDE 90, 5-MeO-BDE 99, 6-MeO-BDE 99, 5-MeO-BDE 100, 4-MeO-BDE 101, 4-MeO-BDE 103, 6-MeO-BDE 140, 3-MeO-BDE 154, 6-MeO-BDE 157. The following compounds, 2,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (Tri-BHD) and 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (Tetra-BHD), synthesized as previously described[9], were obtained at a concentration of 1.6 ng/µL in *iso*-octane from W. Vetter.

Sample preparation: Approximately 0.6 g of pooled fish liver were grounded with sodium sulphate and spiked with 2 ng of BDE 77 used as internal standard. Samples were extracted for 2 hours by hot Soxhlet with a mixture of acetone/hexane (1/3, *v/v*). The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4, 44%), using 50 mL of a mixture of hexane/dichloromethane (DCM) (1/1, *v/v*) for elution of the analytes. The extract was evaporated and a second clean-up step on 1 g Florisil (Supelclean) was carried out, using 12 mL of hexane/DCM (1/1, *v/v*) for elution. The eluate was evaporated to dryness with nitrogen and redissolved in 100 µL of isooctane.

Analysis: An Agilent 6890-5973 GC-MS operated in electron capture negative ionization (ECNI) mode was equipped with a 30 m x 0.25 mm x 0.25 µm DB-5 capillary column. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 mL/min). The electron multiplier voltage was set at 2200 V. One µL of the extract was injected in solvent vent mode (initial injector temperature at 90 °C, stay 0.03 min, then heated with 700 °C/min to 300 °C, vent time 0.03 min, vent flow 75 mL/min, splitless time 1.50 min). The temperature of the DB-5 column was programmed from 90 °C (1.50 min) to 230 °C at a rate of 15 °C/min and then to 300 °C at a rate of 5 °C/min, stay 10 min. Bromine ions (*m/z* = 79 and 81) were acquired in selected ion monitoring (SIM) mode. Dwell times were set at 50 ms. For

the additional confirmatory acquisition of ECNI full scan spectra (*m/z* range 70-650 amu), the GC was operated under the same chromatographic conditions.

For confirmation of MeO-PBDEs and polybrominated hexahydroxanthenes (PBHDs), the extracts were injected into a GC/MS operated in electron ionization (EI) mode and equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The mass spectrometer was used in SIM mode with two most intense ions (typically from the molecular cluster) acquired for each homologue group or isomer. One µL of the extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min, pressure pulse 25 psi, pulse time 1.50 min, splitless time 1.50 min). Helium was used as carrier gas at constant flow (1 mL/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min and kept for 20 min.

Quality Assurance and Quality Control: Quality control was performed by the analysis of four procedural blanks, a replicate sample and a standard reference material (SRM 1945, PCBs and PBDEs in whale blubber). For the replicate and SRM 1945, the relative standard deviations (RSD) were < 10 %. Recoveries of analytes were between 70 and 100 % (RSD < 10 %) as measured by spiking experiments (n = 5) at a concentration of 20 ng/g lipid weight for each individual compound. Additionally, the method performance was assessed through successful participation to interlaboratory studies organized by NIST (PCBs and PBDEs in marine mammals). Procedural blanks of PBDEs were consistent (RSD < 20 %) and therefore the mean value of each analyte in the procedural blanks was used for subtraction. MeO-BDEs and PBHDs were not present in the procedural blanks. After blank subtraction, the limit of quantification (LOQ) was set at 3*SD of the value obtained in the procedural blanks, ensuring > 99 % certainty that the reported value is originating from the sample. Method LOQs ranged from 0.2 to 0.3 ng/g lipid weight (lw) for individual PBDE and MeO-PBDE congeners and were 2 ng/g lw for each PBHD isomer. In agreement with previous reports[9-11], the response factors of PBHDs were 6 to 8 times lower than those of PBDE congeners with the same number of bromine atoms.

Results and discussion

There were differences in the lipid contents of the two species, with the CC species having higher values (Table 1). The values agree with previous data indicating a high lipid percentage in the liver of deep-sea fish species[1,2].

PBDEs. In the CC species, except for BDE 183, all studied PBDE congeners could be measured, with BDE 47 (mean 8.1 ng/g lw) being the dominant congener, followed by BDE 100 (mean 2.8 ng/g lw), BDE 154 (mean 2.4 ng/g lw) and BDE 99 (mean 1.5 ng/g lw) (Table 1, Figure 1). Sum PBDEs ranged between 12.7 and 27.3 ng/g lw for the individual pools, with a mean of 16.9 ± 5.5 ng/g lw. In the TT species, only PBDE congeners 28, 49, 47 and 100 could be measured, with BDE 47 (mean 2.4 ng/g lw) being the dominant congener, followed by BDE 100 (mean 1.0 ng/g lw). Sum PBDEs ranged between 3.2 and 7.0 ng/g lw for the individual pools, with a mean of 4.5 ± 1.1 ng/g lw, which is approximately 4 times lower than in the CC species.

The differences in the PBDE concentrations observed between the two deep-sea fish could not be related to the trophic level, as both species have been reported to feed on copepods, polychaetes or fish. However, the differences might be due to their feeding habits, as the TT species are reported to be pelagic predatory feeders, while the CC species are primarily benthic feeders. Since a vertical gradient in the concentrations of hydrophobic contaminants has been evidenced in the water column[4], it is possible that benthic feeders, which are in close contact with contaminated sediments, could be exposed to higher levels of contaminants than pelagic feeders. For both species, no relationship was observed between the PBDE concentrations in the different pools and mean lengths of the fishes constituting the pools.

The detection of PBDEs in deep-sea fishes confirms that PBDEs are transportable to the deep ocean in a similar way to that evidenced for other hydrophobic contaminants. The PBDE concentrations in the deep-sea fish from the Mediterranean Sea were higher that the mean levels of 1.5 ng/g lw measured in deep-sea fishes from the Sulu Sea[3]. The PBDE concentrations in the present study are lower than those found by Sinkkonen et al.[6] in Baltic salmon muscle (sum PBDE 49.5 ng/g lw) and similar to those found by Gomara et al.[12] in edible fish from the Mediterranean Sea, with the median concentration of total PBDEs of 3.2 ng/g lw.

MeO-PBDEs. Although a large number of mono- to hexabrominated) MeO-PBDE congeners were analysed, only 2 tetrabrominated congeners (2'-MeO-BDE 68 and 6-MeO-BDE 47) could be consistently measured above LOQ in both species (Table 1). This is in agreement with various reports of MeO-PBDEs present in marine fish[6,7], which indicated that MeO-tetraBDEs are present at much higher levels than their tri- or pentabrominated homologues. These compounds have been reported as having natural origin[13], being formed by sponges, bacteria or algae.

Table 1. Concentrations of major organobrominated compounds (ng/g lw) in two species of deep-sea fish from the Mediterranean Sea. Values below LOQ were replaced by ½*LOQ.

		C. coelorhynchus (CC)		T. trachyrinchus (TT)	
	LOQ	**Mean**	**SD**	**Mean**	**SD**
Lipids (%)		41.2	5.7	26.7	4.5
BDE 28	0.2	0.2	0.0	0.3	0.1
BDE 49	0.2	0.7	0.2	0.7	0.2
BDE 47	0.2	8.1	3.2	2.4	0.7
BDE 66	0.2	0.7	0.4	< LOQ	
BDE 100	0.2	2.8	0.8	1.1	0.4
BDE 99	0.2	1.5	0.5	< LOQ	
BDE 154	0.3	2.4	0.6	< LOQ	
BDE 153	0.3	0.4	0.2	< LOQ	
Sum PBDEs		16.9	5.5	4.5	1.1
2'-MeO-BDE 68	0.2	3.4	1.3	2.8	0.5
6-MeO-BDE 47	0.2	25.5	7.5	3.7	0.7
Sum MeO-PBDEs		28.9	8.2	6.5	1.0
Tri-BHD	2	180	30	65	12
Tetra-BHD	2	350	60	7000	5100
Sum PBHDs		530	80	7000	5100
CB 153	1	320	130	3500	1600
Sum PCBs		1200	450	12300	5900

Similar to PBDEs, the sum of MeO-PBDEs was higher in the CC species (mean 28.9, range 22.5 - 39.0 ng/g lw) compared to the TT species (mean 6.5, range 4.8 - 8.8 ng/g lw). In the CC species, 6-MeO-BDE 47 was present at higher levels (mean 25.5 ng/g lw) than 2'-MeO-BDE 68 (mean 3.4 ng/g lw), while in the TT species, both congeners had similar concentrations (3.7 and 2.8 ng/g lw) (Table 1). The differences in the ratios between 6-MeO-BDE 47 and 2'-MeO-BDE 68 are probably due to variability in the production of these two congeners from natural sources, in their availability in the prey species, in their uptake or their biotransformation rate. The higher proportion of 6-MeO-BDE 47 in our fish samples agrees with reports from the Northern Hemisphere, where 6-MeO-BDE 47 has been found to be the dominant congener in fish and marine mammals[6-8,14,15].

Although the sources of these two classes of brominated compounds are different, the concentrations of sum MeO-PBDEs in both fish species were in the same range and had the same trend in variation as the concentrations of sum PBDEs.

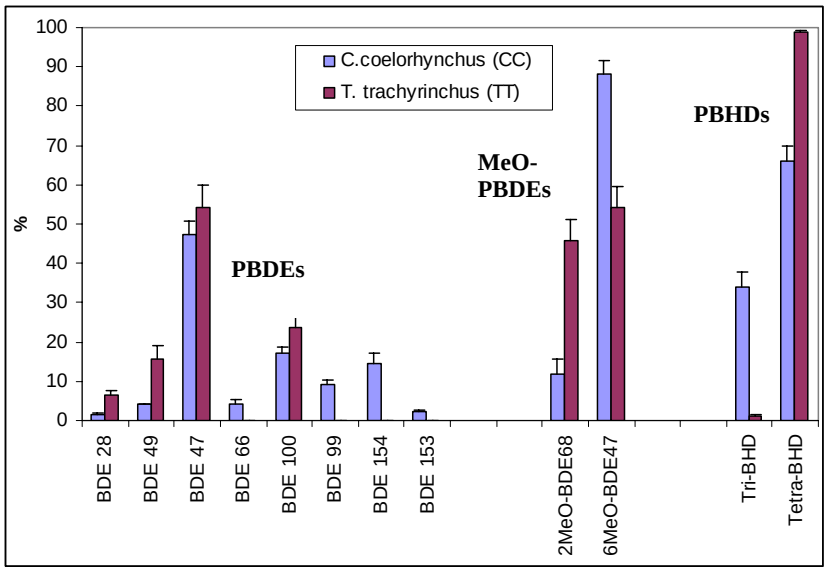
PBHDs. Interestingly, two isomers of another class of brominated natural products - polybrominated hexahydroxanthenes (PBHDs) - were identified in all analyzed fish. These two compounds (tri-BHD and tetra-BHD) were only recently reported as naturally occurring brominated compounds[9-11]. Concentrations of these two isomers and their ratio had a large variation between the two deep-sea fish species. Moreover, their distribution was also different from that of PBDEs and MeO-PBDEs, with higher concentrations.

Concentrations found in the CC species were slightly higher for tetra-BHD (mean 350 ng/g lw) than for tri-BHD (mean 180 ng/g lw). However, for the TT species, the concentrations of tri-BHD (mean 65 ng/g lw) were much lower than the concentrations of tetra-BHD (mean 7000 ng/g lw). In these species, the tetra-BHD isomer made up 98% of the total PHBD concentration. This special profile has not been reported until now and it differs

dramatically from what has been detected so far in fish muscle[9,10] and fish oils[11], in which similar concentrations were found for tri-BHD and tetra-BHD or slightly higher for tri-BHD.

The concentrations of PBHDs in both species were higher than those of MeO-PBDEs or PBDEs, but were in the same order of magnitude as PCBs (Table 1). Concentrations of PBHDs in the present fish species were higher than those found in fish oil dietary supplements[11], (maximum 8.0 and 11.6 ng/g oil for tri-BHD and tetra-BHD, respectively). However, they were in the range of concentrations found in muscle fish (between <5 and 1000 ng/g lw for tri-BHD and between <5 and 560 ng/g lw for tetra-BHD) from different locations world-wide[9].

Figure 1. Percentage contribution to the sum of PBDEs, MeO-PBDEs and PBHDs for each of the isomers consistently detected in the two species of deep-sea fish.



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