NATURALLY-OCCURRING AND ANTHROPOGENIC ORGANOBROMINATED COMPOUNDS IN MARINE SPECIES FROM SYDNEY HARBOUR, AUSTRALIA

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Introduction

Historically, much of the industry in New South Wales (Australia), including chemical manufacturing, was located around Sydney Harbour and its tributaries[1]. As a result, previous studies have found significant levels PCDDs/PCDFs, PCBs and other chlorinated hydrocarbons in fish, crustaceans and molluscs[2-4]. However, the presence of newer contaminants, such as polybrominated diphenyl ethers (PBDEs), in Australian marine environment, and more specific in the Sydney Harbour has not yet been investigated. Additionally, naturally-occurring organohalogenated compounds (e.g. MeO-PBDEs[5]), have been reported in Australian marine environment[6-8], but not investigated in details. The present work focussed on various species representative for the marine fauna of Sydney Harbour. We investigated the concentrations and profiles of PBDEs contaminants in these species and, we also evaluated the presence of naturally-occurring organobromines.

Materials and Methods

Location and samples: Eight species including fish, crustacea and mollusc were sampled in the eastern part of Sydney Harbour a marine dominant estuary[9] located in New South Wales, Australia (Figure 1). The organisms were sampled using commercial fishing methods including trawling, gill or seine netting. The fish were immediately transferred to the laboratory and frozen at -20 °C until sub-sampling. Composite samples of muscle tissue were taken from 10 adult individuals of each fish or crab species and freeze dried prior to analysis.

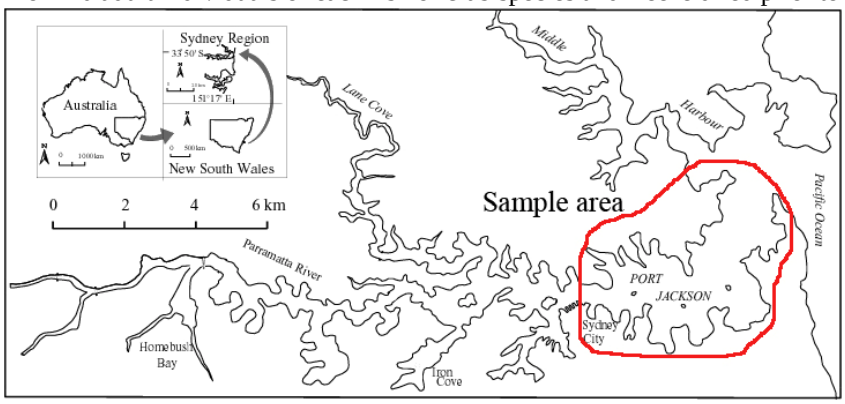


Figure 1. Map of Sydney Harbour showing the area from which samples were taken.

The species selected represent differing trophic levels and include six fish species, one crustacean and one mollusc (Figure 2). The fish included flounder (*Pseudorhombus jenynsii*), tailor (*Pomatomus saltator*), yellowfin bream (*Acanthopagrus australis*), fanbelly leatherjacket (*Monocanthus chinensis*), sea mullet (*Mugil cephalus*) and luderick (*Girella tricuspidata*). The crustacean species were the blue swimmer crab *(Portunus peligicus*) and the squid, Loligo squid (*Loligo* sp). Differences in trophic level were confirmed using stable isotope analysis.
Materials: Standards of PBDEs (IUPAC no. 28, 49, 47, 66, 85, 99, 100, 153, 154 and 183) and MeO-PBDEs 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 (triBHD) and 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (tetraBHD), isolated as previously described[10], were obtained at a concentration of 1.6 ng/µL in toluene from W. Vetter.

Sample preparation: Between 3 and 6 g of freeze-dried tissue (depending on the lipid content) were 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/*n*-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 *n*-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 *n*-hexane/DCM (1/1, *v/v*) for elution. The eluate was evaporated to dryness with nitrogen and re-disolved in 100 µL of *iso*-octane.

Analysis: An Agilent 6890-5973 GC-MS operated in electron capture negative ion (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. One µL of the extract was injected in solvent vent mode (splitless time 1.50 min). Bromine isotope 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. The ion source, quadrupole and interface temperatures were 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. Two µL extract were injected in cold pulsed splitless mode (splitless time 1.50 min).

Stable isotopes: Ground and dried samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ using a continuous flow-isotope ratio mass spectrometer (Micromass Isoprime EuroVector EA300, Manchester, UK). Stable isotope ratios of samples ($\delta^{13}C$ or $\delta^{15}N$ values [‰]) were assessed against the reference standards ANU sucrose for $\delta^{13}C$ values [‰] and atmospheric N_2 for $\delta^{15}N$ values [‰]. Isotope ratios are expressed as either $\delta^{13}C$ or $\delta^{15}N$ and relate to the ratios $^{13}C/^{12}C$ and $^{14}N/^{15}N$, respectively.

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 (lw). 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 lw for individual PBDE and MeO-PBDE congeners and were 2 ng/g lw for each PBHD isomer. In agreement with previous reports[10-12], 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

PBDEs. In general, profiles were dominated by BDE 47 in all species (Table 1). BDE 47 contributed at a highest percentage (between 55 and 68% of the total PBDEs) in flounder, bream and tailor, which are at a higher trophic level as determined by $\delta^{15}N$ measurements (Figure 2). BDE 100 was the second dominant congener, with the exception of fanbelly leatherjacket and swimmer crab, for which concentrations of BDE 100 were similar to BDE 99. The PBDE concentrations were broadly related to trophic position, with the highest concentrations found in tailor, which is considered to be largely piscivorous. Fish species with omnivorous/herbivorous (fanbelly leatherjacket and luderick) or detritivorous (sea mullet) diets had lower lipid-normalized concentrations of PBDEs. The blue swimmer crab and squid had total PBDE concentrations considerably lower than the other species. Blue swimmer crab is largely detritivorous, but also an opportunistic carnivore. The squid probably

consumes similar prey as the fish species and, subsequently, they have also been found to accumulate dioxin-like compounds at concentrations comparable with fish[4]. These results indicate that these molluscs display some resistance to accumulation or a capacity to eliminate PBDEs, which is not present in fish.

Table 1. Concentrations of major organobromine compounds (ng/g lw) in various marine species from the Sydney Harbour (Australia). Values in brackets represent standard deviations. Values below LOQ were replaced by ½*LOQ. The following species were investigated: *P. jenynsii* (FLO), *P. saltator* (TAI), *A. australis* (BRE), *M. chinensis* (FBL), *Loligo* sp (SQU), *P. peligicus* (BSC), *M. cephalus* (SMU), *G. tricuspidata* (LUD).

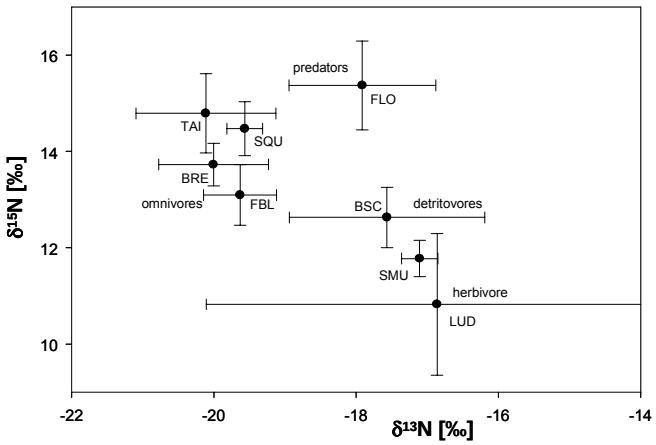
	LOQ	FLO (n=4)	TAI (n=4)	BRE (n=5)	FBL (n=4)	SQU (n=3)	BSC (n=3)	SMU (n=5)	LUD (n=5)
BDE 28	0.2	1.7	1.1	2.0	3.8	0.6	0.2	1.4	0.6
BDE 49	0.2	2.0	5.5	1.7	0.3	<LOQ	<LOQ	1.3	0.6
BDE 47	0.2	50.1	71.6	60.0	13.2	3.4	11.1	21.1	19.7
BDE 66	0.2	1.3	1.3	1.7	0.6	0.3	1.1	0.5	1.0
BDE 100	0.2	10.8	15.0	15.3	2.4	0.8	1.5	4.7	4.7
BDE 99	0.2	4.4	7.3	1.4	2.4	<LOQ	1.9	0.8	1.0
BDE 85	0.2	0.7	0.8	0.9	0.3	<LOQ	<LOQ	0.6	0.3
BDE 154	0.3	2.7	3.4	3.7	0.5	0.4	<LOQ	1.7	1.1
BDE 153	0.3	1.2	1.6	1.6	0.3	<LOQ	<LOQ	0.7	0.6
Sum PBDEs		**74.8 (8.5)**	**107.2 (41.3)**	**88.4 (44.5)**	**23.9 (5.0)**	**5.8 (1.5)**	**16.4 (3.8)**	**32.9 (12.6)**	**29.6 (24.0)**
Total HBCD	1.5	**<LOQ**	**1.9**	**1.7**	**3.3**	**<LOQ**	**<LOQ**	**2.8**	**<LOQ**
2,4,6-tribromo-anisole TBA	0.2	**17.1 (7.7)**	**11.7 (5.9)**	**28.4 (3.1)**	**26.3 (27.8)**	**13.3 (4.1)**	**29.3 (34.6)**	**64.9 (75.8)**	**12.0 (3.5)**
2'-MeO-BDE 68	0.2	22.4	66.5	0.2	0.2	5.1	0.5	8.6	16.9
6-MeO-BDE 47	0.2	18.9	43.6	10.6	0.5	4.7	1.8	6.1	18.9
Sum MeO-PBDEs		**41.3 (51.6)**	**110.1 (13.9)**	**10.8 (4.3)**	**0.7 (0.6)**	**9.9 (5.6)**	**2.3 (1.1)**	**14.7 (17.8)**	**35.8 (17.1**
Tri-BHD	2	29.6	90.4	12.3	3.7	19.1	11.5	35.7	8.9
Tetra-BHD	2	19.9	55.6	5.8	<LOQ	47.5	<LOQ	14.6	22.3
Sum PBHDs		**49.5 (24.8)**	**146.0 (34.6)**	**18.1 (7.6)**	**4.7 (3.5)**	**66.6 (38.1)**	**13.0 (12.8)**	**50.4 (70.9)**	**31.2 (15.4)**

MeO-PBDEs. Although a large number of mono- to hexabrominated MeO-PBDE congeners were analyzed, only two tetrabrominated congeners (2'-MeO-BDE 68 and 6-MeO-BDE 47) could be consistently measured above LOQ in all species (Table 1). This is in agreement with various reports in marine fish[13,14], which indicated that MeO-tetraBDEs are present at much higher levels than their tri- or pentabrominated homologues. Both 2'-MeO-BDE 68 and 6-MeO-BDE 47 had higher concentrations in flounder and tailor, species from the higher trophic levels. Fanbelly leatherjacket and luderick are known to feed on various macroalgae, a potential source of MeO-PBDEs, but showed large variation in concentration: luderick shows levels comparable with flounder, whereas those in fanbelly leatherjacket were the lowest observed. All eight species had different ratios of 2'-MeO-BDE 68 and 6-MeO-BDE 47, but yellowfin bream displayed the greatest variation (Table 1). There is no outstanding life history characteristic which could explain this degree of variation. More generally, the differences among species were 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. In the present study, 2'-MeO-BDE 68 was found at similar or slightly higher concentrations than 6-MeO-BDE 47. This agrees with reports in mammals and fish from Australia[6-8], where 2'-MeO-BDE 68 was in higher proportion.

PBHDs. Interestingly, two isomers of another class of brominated natural products - polybrominated hexahydroxanthenes (PBHDs) - were identified in most of the analyzed samples. These two compounds (triBHD and tetraBHD) were only recently reported as naturally occurring brominated compound[10-12]. Concentrations of these two isomers and their ratio had a large variation between the investigated species. Tailor had the highest

concentrations of total PBHDs followed by squid, sea mullet and flounder (in this order) with similar concentrations. It is notable that squid had higher concentrations of tetraBHD compared to triBHD, which is in contrast to all species except by luderick. Concentrations of PBHDs in the present fish were higher than those found in fish oil dietary supplements[12], (maximum 8.0 and 11.6 ng/g oil for triBHD and tetraBHD, respectively). However, they were in the range of concentrations found in fish (between <5 and 1000 ng/g lw for triBHD and between <5 and 560 ng/g lw for tetraBHD) from different locations world-wide[10].

Figure 2. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values [‰]) of the investigated marine species.



Among the organobromines analysed for this study, PBDEs exhibited the strongest relationship with trophic level, with the three most carnivorous fish species displaying the highest concentrations. Trophic relationships were not as evident for MeO-PBDEs and PBHDs: the highest concentrations of MeO-PBDEs were found in only two of these fish species and for PBHDs, only one fish species had markedly higher concentrations, while squid had high levels of tetraBHD.

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