HALOGENATED NATURAL PRODUCTS (HNPs) IN FISH, SEA CUCUMBER AND SEDIMENT FROM THE GREAT BARRIER REEF (AUSTRALIA)

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Introduction

Several thousand different halogenated natural products have been discovered to date [1]. A few of them share the properties (lipophilicity and persistence) of persistent organic pollutants (POPs) which makes them to environmentally relevant compounds [2]. The Great Barrier Reef, along the northeast coast of Australia, was shown to be a rich source of halogenated natural products (HNPs) [3,4]. Particularly high levels (up to mg/kg lipids) of some HNPs were determined in marine mammals from this area [5,6]. Chemical structures of these HNPs may be structurally very similar to anthropogenic contaminants. For instance, the tetrabrominated HNPs 6-MeO-BDE-47 (BC-3, **Figure 1a**) 2'-MeO-BDE-68 (BC-2, **Figure 1b**), and 2,6´-diMeO-BDE-68 (BC-11, **Figure 1f**) differ from the anthropogenic polybrominated diphenyl ethers (PBDEs), which are heavily used as brominated flame retardants (BFRs), only in the form of one or two additional $-OCH_3$ groups in *ortho*-positions. By contrast, the *N*-heterocyclic heptahalogenated-1'-methyl-1,2'-bipyrroles [6] (e.g. Q1, **Figure 1d**), hexahalogenated-1,1'-dimethyl-2,2'-bipyrroles [7] and the tetrabromo-1-methylpyrrole have no related anthropogenic analogues but the overall appearance of the fully halogenated backbone was similar to compounds like PCBs. While several studies reported on HNP residues in marine mammals from the Great Barrier Reef, data on fish and lower trophic level biota are scarce. For this reason we quantified HNPs in fish and a sea cucumber and compared the levels and patterns with those found in sediment and passive water samples.

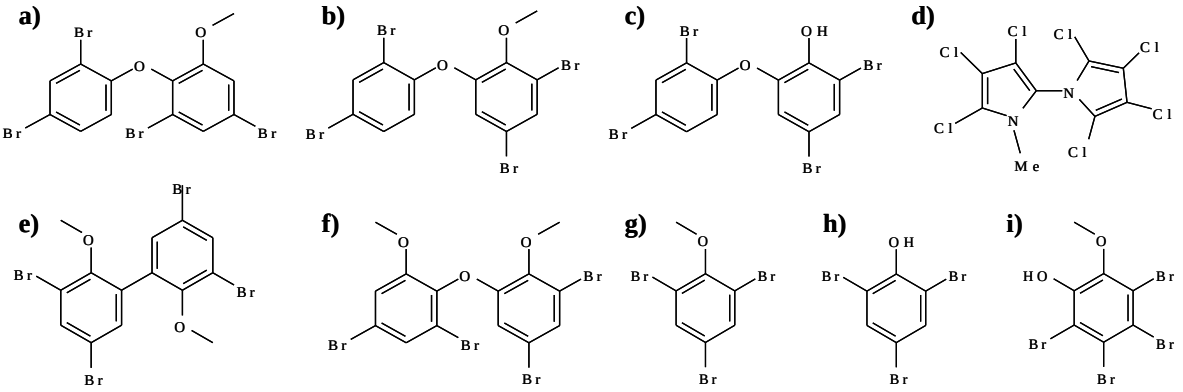


Figure 1: Structures of polyhalogenated natural products (HNPs): (a) 6-MeO-BDE-47, (b) 2'-MeO-BDE-68 (BC-2), (c) 2'-OH-BDE-68, (d) 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1), (e) 2,2'-diMeO-BB-80 (BC-1), (f) 2',6-diMeO-BDE 68 (BC-11), (g) 2,4,6-tibromoanisole (TBA), (h) 2,4,6-tribromophenol (TBP), (i) tetrabromoguaiacol

Materials and methods

Chemicals. *Iso*-octane (for residue analysis), *n*-hexane (for residue analysis), cyclohexane (>99%) and ethyl acetate (>99%) and silica gel 60 were purchased from Sigma Aldrich (Steinheim, Germany) supplied by Fluka, and cyclohexane/ethyl acetate were purified by aceotropic distillation. The origin of the reference standards were reported elsewhere [8] i.e. 2,4,6-tribromoanisole (2,4,6-TBA, **Figure 1g**), 2,4-dibromophenol (2,4-DBP), 2,4,6-tribromophenol (2,4,6-TBP, **Figure 1h**), (1*R*,2*S*,4*R*,5*R*,1'*E*)-2-bromo-1-bromomethyl-1,4-dichloro-5-(2'-chloroethenyl)-5-methylcyclo-hexane (MHC-1), 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1),

hexabromobenzene, BDE-47, 2,2'-diMeO-BB-80 (BC-1, **Figure 1e**), 2'-MeO-BDE-68 (BC-2), 6-MeO-BDE-47 (BC-3), 5,5'-dichloro-1,1'-dimethyl-3,3',4,4'-tetrabromo-2,2'-bipyrrole (BC-10), 2',6-diMeO-BDE-68 (BC-11), 2,7-dibromo-4a-bromo-methyl-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), named in elution order on an HP-5MS column. Additional standards were synthesized in our laboratory: 2'-OH-BDE-68 according to Francesconi & Ghisalberti [9], 6'-MeO-BDE-66 [10] and perdeuterated α-HCH [11].

Extraction and cleanup. Extraction of freeze dried samples was performed with accelerated solvent extraction with an ASE 200 system (Dionex, Sunnyvale, CA, USA) using ethyl acetate/cyclohexane (54:46, w/w) as the solvent. Lipids were removed by gel permeation chromatography (GPC) by means of a Gilson (Limburg Germany) system (sampling injector 231 XL, syringe pump 402, pump 305, manometric module 806 and fraction collector 206) employing a Bio-Beads SX-3 (41 x 2.5 cm internal diameter) column operated with a flow of 5 mL/min ethyl acetate/cyclohexane (54:46, w/w) collecting the target fraction from 22-55 min. Residual lipids were finally removed with column chromatography after solvent exchange to *iso*-octane using a 3 g column of 30% deactivated silica gel and eluting with 60 mL *n*-hexane [12]. Perdeuterated α-HCH was used as cleanup standard and 6'-MeO-BDE-66 was used as syringe spike.

Gas chromatography mass spectrometry (GC/MS). A 7890/5975c GC/MS system form Agilent (Waldbronn, Germany) was used for GC/ECNI-MS measurements. Injections of 1 µL were performed in the pulsed splitless mode with a programmed temperature vaporizer (80 °C, held for 0.01 min, then at 500 °C/min to 300 °C, held for 2 min, and finally at 10 °C/min to 260 °C until the end of the run). An HP-5MS column (30 m, 0.25 mm i.d., 0.25 µm d_f) was used in combination with the following GC oven program: 50 °C (1 min), then at 10 °C/min to 300 °C (19 min). Helium 5.0 was used as the carrier gas with a constant flow of 1.2 mL/min and methane 5.5 was used as reagent gas at 1.6×10^{-4} torr. In the selected ion monitoring mode (SIM), where the transfer line, quadrupole and ion source were kept at 300 °C, 150 °C and 150°C, respectively, we recorded *m/z* 35, *m/z* 37, *m/z* 72, *m/z* 79, *m/z* 81, *m/z* 114, *m/z* 116, *m/z* 158, *m/z* 159, *m/z* 160, *m/z* 161, *m/z* 386 and *m/z* 388. In the full scan mode, *m/z* 30-800 were recorded.
GC/EI-MS analyses were performed with a 6890/5973 GC/MS system (Hewlett Packard, Waldbronn, Germany) in the SIM mode using *m/z* 498, *m/z* 500, *m/z* 502, *m/z* 504, *m/z* 514 and *m/z* 516. The GC conditions were just as above, except that a spit/splitless injector was used at 250 °C in the splitless mode for the quantification of 2'-OH-BDE-68.

Origin of samples. Fish (of an unknown, but edible species; n=2) and sea cucumber (n=1) samples were collected offshore Heron Island (southern Great Barrier Reef, Australia). Fish samples were dissected into liver, filet and gills (only fish II) which were analyzed separately. The sediment sample (600 g) was from the Whitsunday Island region while the passive water sampler was deployed offshore North Keppel Island (southern Great Barrier Reef) and prepared according to Vetter et al.[3].

Results and discussion

The chromatograms obtained from the samples analyzed were dominated by HNPs while PBDEs, polychlorinated biphenyls (PCBs) and other anthopogenic compounds were only detected at blank levels. The fish samples were clearly dominated by 2'-MeO-BDE-68 (BC-2) (**Table 1**) which was also very frequently detected in passive water samples from the Great Barrier Reef where BC-2 showed the second highest detection frequency only exceeded by TBA [3]. The ratio of BC-2/BC-3 was basically the same in fish filet and liver, but twice as high in the gills of fish II. This may indicate that there was a high exposure of BC-2 through the water phase. The ratios observed were in agreement with the ones observed in marine mammals from this area and the concentrations were comparable, too [5]. This is remarkable because fish are located on a lower trophic level of the marine food chain than marine mammals. Q1 (**Figure 1d**) was also detected in all fish tissues and the distribution in the fish organs was relatively homogenous. The dibromotrichloromonoterpene MHC-1 was detected for the first time in environmental samples from the Great Barrier Reef. Noteworthy as well, fish I also contained the phenolic 2'-OH-BDE-68. This HNP differs from 2'-MeO-BDE-68 (BC-2) only in the lack of the methyl group (compare **Figures 1b,c**). The concentrations of the 2'-OH-BDE-68 reached only 0.3% (in liver)

and 0.1% (in filet) of the BC-2 amount (**Table 1**). 2'-OH-BDE-68 was also detected in the passive sampler extracts.

Table 1: Organohalogen levels detected in two fish samples from Heron Island (Great Barrier Reef)

	Fish I			**Fish II**		
	Liver	Filet		Liver	Filet	Gills
Lipid [%] of d.w.	21	3		11.7	1.6	18
	Organohalogen levels [ng/g lipid weight]					
2,4-dibromophenol (2,4-DBP)	273	18.4		118	n.d.	10.9
2,4,6-tribromoanisole (2,4,6-TBA)		10.2		1.7	10.5	7
2,4,6-tribromophenol (2,4,6-TBP)	1580	n.d.		3453	n.d.	188
MHC-1	5.2	5.9		2.1	5.4	4.8
Q1	27.3	29.6		79.4	156	110
BC-2	5968	2436		825	1088	1844
BC-1	117	44.5		25.1	27.3	31.1
BC-3	257	138		115	140	130
2'-OH-BDE-68	20	3		n.d.	n.d.	n.d.
BC-11	15.3	12.8		8	28.5	10.8
Ratio BC-2/BC-3	23.2	17.6		7.2	7.8	14.2

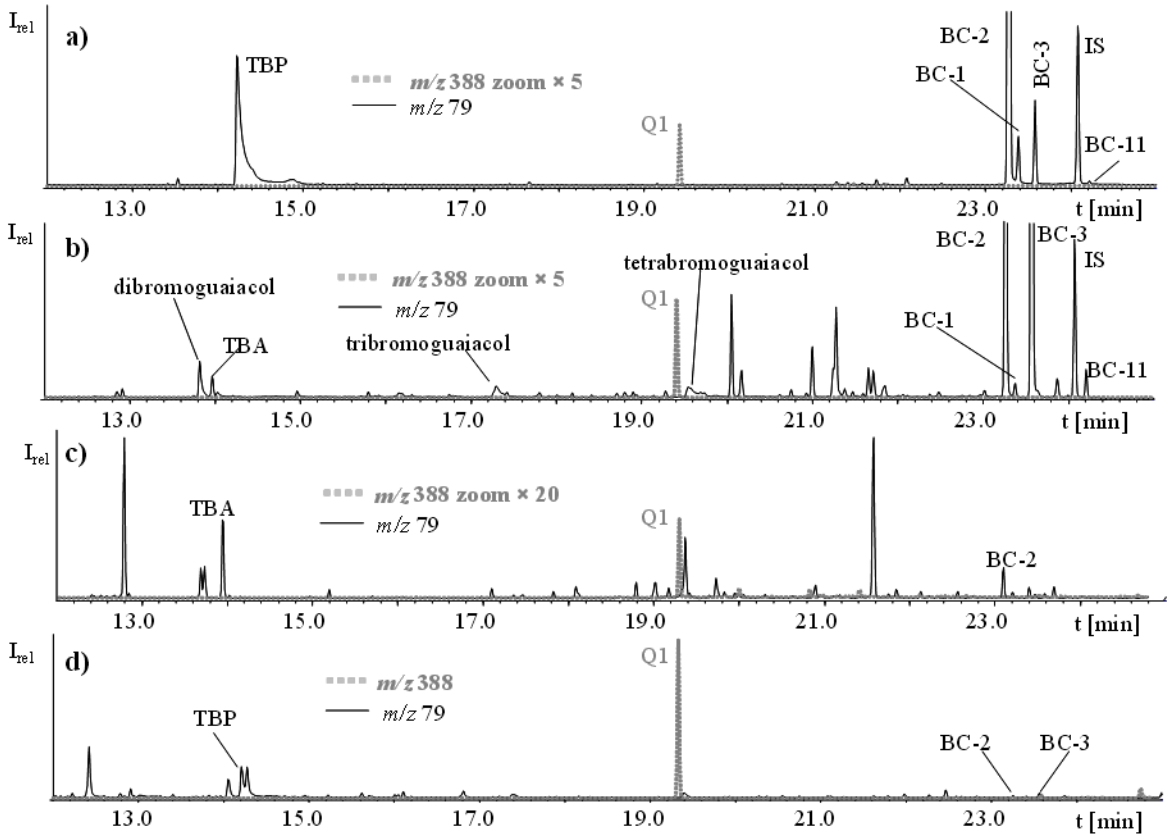
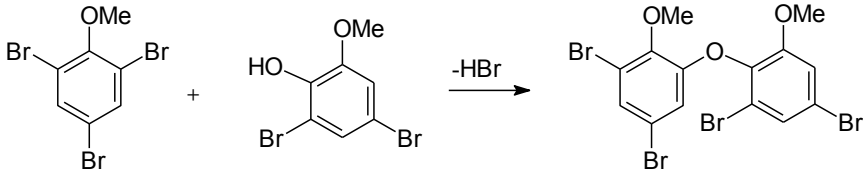


Figure 2: GC/ECNI-MS-SIM chromatograms (*m/z* 79 and *m/z* 388) of samples of purified (a) fish liver (fish I), (b) sea cucumber and (c) passive water sampler from North Keppel Island and (d) sediment from the Whitsunday Islands. Chromatograms (a) and (b) were scaled that the IS was 100%

A high abundance of phenolic compounds in the liver was also observed for bromophenols (**Table 1**). The 2,4,6-tribromophenol (TBP) was found in the ppm-level in liver while the concentration was at least one order of magnitude lower in filet of both fishes. The same proportions were also determined for 2,4-DBP. By contrast, 2,4,6-tribromoanisole (TBA) had a much higher concentration in the filet (**Table 1**).
The GC/ECNI-MS pattern of the sea cucumber sample (**Figure 2b**) was characterized by even more organobromine peaks than the fish samples. Three tailing peaks originating from organobromine peaks in the sea cucumber were not present in the fish. GC/ECNI-MS in full scan mode was used to characterize them as di-, tri- and tetrabromoguaiacole (**Figure 1i**) isomers. The bromoguaiacols, which were detected for the first time in biota from Australia, could be the monomeric precursors of the dimethoxydiphenyl ether BC-11 (**Figure 3**), similarly to bromophenols (2 units) being the precursors of BC-2 and BC-3 [2].



2,4,6-tribromoanisole + 4,6-dibromoguaiacol → BC-11 (2,6´-diMeO-BDE 68)

Figure 3: Potential natural formation of BC-11

This sample also contained relatively high amounts of tribromophenoxyanisoles. Although the dominance of BC-2 (and less pronounced BC-3) was evident, the pattern in the sea cucumber was more similar to the passive sampler extract (although this did not contain the bromoguaiacols). Which showed a higher complexity in the retention time range between 2,4,6-TBP and BC-2 (**Figure 2a-c**). Q1 marked an abundant peak in all samples including sediment (**Figure 2**). The polychlorinated alkaloid Q1 was the most abundant peak in the sediment (**Figure 2d**). The concentration of Q1 in sediment was 2.5 ng/g dry weight. Noteworthy, Q1 had not been detected before in sediment from the Great Barrier Reef.
Although the samples originated from the same habitat, the HNP pattern was rather unique in the different matrices. The high abundance of TBP in fish liver suggested uptake via feed and not the water phase because the concentration in the passive water samples was much lower. In addition, bromoguaiacols were only detected in the sea cucumber and neither in fish nor in the passive water samples. Local and seasonal differences in the production and release of HNPs could be the reason for the different HNP patterns. Our results indicate that there is need for a more thorough analysis of lower trophic biota on HNPs in order to better understand the food web enrichment of HNPs and the high concentrations of some HNPs in marine mammals. Such future studies should include a higher sample size and more species. In general, the occurrence and dimension of HNPs in the environment appears to be much less predictable compared with anthropogenic POPs.

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