HALOGENATED NATURAL PRODUCTS AND PCBs IN SQUID FROM SOUTH AFRICA´S OCEANS

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Introduction

In addition to man-made persistent organic pollutants (POPs), several halogenated natural products (HNPs) - examples are shown in **Fig. 1** - have been detected at high abundance in marine organisms.[1] Some HNPs are structurally related to anthropogenic PBDEs. Consequently, HNPs were recently (2016) ranked as emerging contaminants by the Arctic Monitoring and Assessment Programme (AMAP).[2] However, the occurrence of HNPs is difficult to predict and differs from the environmental distribution of POPs.[3] In addition, information on HNPs is frequently not available. So far, little information existed on the occurrence of HNPs in marine regions of Africa. In this context, South Africa is particularly interesting because the country embodies the natural demarcation between the South Atlantic Ocean in the West and Indian Ocean in the East. For both reasons, we were interested in the analysis of marine samples from different sites along the coast of South Africa. In this study squid (*Loligo reynaudii*) samples were taken at three different sites in South Africa and analysed by GC/ECNI-MS.

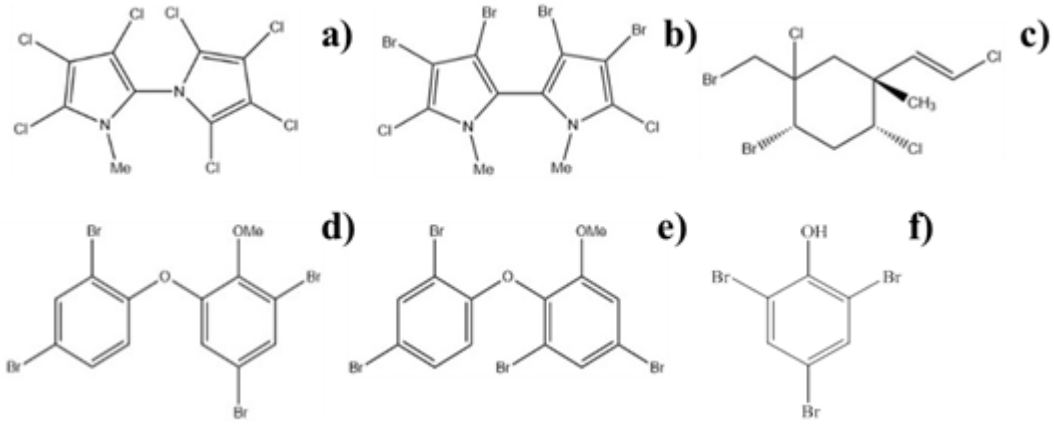

Figure 1: Structures of relevant halogenated natural products (HNPs) with (a) 2,3,3´,4,4´,5,5´-heptachloro-1´-methyl-1,2´-bipyrrole (Q1), (b) 5,5´-dichloro-3,3´,4,4´-tetrabromo-1,1`-dimethyl-2,2`-dipyrrole (BC-10), (c) mixed halogenated compound 1 (MHC-1), (d) 2´-MeO-BDE 68 (BC-2), (e) 6-MeO-BDE 47 (BC-3) and (f) 2,4,6-tribromophenol (2,4,6-TBP).

Materials and methods

Samples. Twenty-five squids (*L. reynaudii*) were collected at three sites in South Africa (**Fig. 2**). Site I was located in the Indian Ocean, site A in the South Atlantic Ocean and site X near the demarcation point between the South Atlantic Ocean and the Indian Ocean. After harvesting, samples were cut and freeze-dried, afterwards stored in plastic tubes at -20 °C until sample clean-up. Squids are obligatory carnivorous species living in the upper middle layer of the shallow sea.[4]

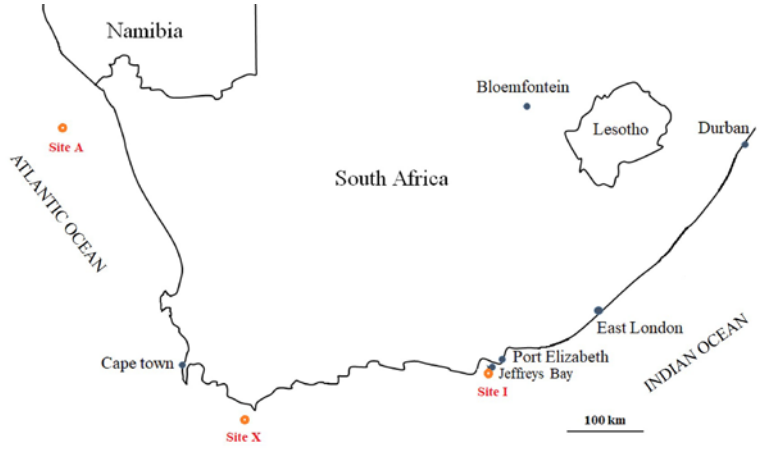

Figure 2: Map of South Africa with the sampling sites (A, X, and I)

Sample cleanup. Samples (~2 g accurately weighed, homogenized freeze-dried squid) was analyzed by accelerated solvent extraction (ASE), gel permeation chromatography (GPC) and adsorption chromatography (silica gel 60, deactivated with 30% water) according to Vetter *et al.*[5] Additionally, a separation of PCBs from organobromine compounds was performed.[5]

GC/ECNI-MS analysis. An Agilent 7890/5975C GC/ECNI-MS system was used with the setup of Bendig *et al.*[6] A DB-5 ultra-inert (UI) column (30 m length x 0.25 mm internal diameter x 0.25 µm film thickness, Agilent, Waldbronn, Germany) was installed in the GC oven.

Results and discussion

Concentrations of PCBs, PBDEs and other POPs.

PCB 153, PCB 138, PCB 180 and PCB 118 were detected and quantified in all squid samples (ΣPCBs). ΣPCBs contamination became lower from East to West: The mean ΣPCB level decreased from 9.3 ng/g lipids (site I, Indian Ocean) over 8.5 ng/g lipids (site X, between the two oceans) to 4.7 ng/g lipids (site A, South Atlantic Ocean) (**Table 1**). PCB 153 was more abundant than PCB 138 in all squid samples at site I on the Indian Ocean (closer to Asia). However, PCB 138 was higher concentrated in virtually all samples from site A (located in the South Atlantic Ocean) and site X (between both oceans). Yet, higher concentrations of PCB 138 at sites X and A were unusual and could not be explained. This observation indicated differences between the three sites.
Munschy *et al.* (2016) found 7.1 ng/g lipids PCBs in albacore tuna (*Thunnus alalunga*) close to site X.[7] African penguin eggs near site I had 48 ng/g lipids PCBs and those near site X had 32 ng/g lipids, respectively.[8] These concentrations are one order of magnitude higher than in squid which is part of the prey of penguins. The studies mentioned above support the notion that aquatic biota from the Indian Ocean along the coast of South Africa seems to be more polluted with PCBs than from the South Atlantic Ocean. Concentrations of ΣPBDEs at sites I/X/A were on average ~35/18/45% of ΣPCB (**Table 1**), with lowest relative PBDE abundance at site X. Even lower concentrations were determined for HCB, HCH and DDT (the latter two are not shown in **Table 1**).

Table 1. Concentration (ng/g lipids) range (mean value) of HNPs and POPs in squids (*Loligo reynaudii*) from three sites of South Africa

#		Site I	Site X	Site A
	HNPs			
1	Q1	33-210 (100)	42-230 (96)	27-85 (43)
2	2,4,6-TBP	1.7-52 (17)	2.0-29 (17)	13-47 (28)
3	BC-3	4.4-7.4 (6.1)	1.6-7.2 (3.4)	1.2-2.2 (1.5)
4	BC-2	<LOD-9.3 (4.6)	<LOD-5.0 (3.0)	<LOD-1.4 (0.25)
5	2,4-DBA	<LOD-14 (2.7)	<LOD-7.4 (4.1)	6.0-30 (14)
6	2,4-DBP	<LOD-7.9 (1.4)	1.5-6.2 (3.8)	0.88-2.6 (1.7)
7	2,4,6-TBA	0.69-2.6 (1.4)	0.33-0.61 (0.48)	0.38-0.74 (0.50)
8	BC-10	<LOD-1.6 (0.88)	0.30-2.4 (1.2)	0.96-3.1 (2.0)
9	MHC-1	0.46-1.7 (0.82)	0.06-0.73 (0.34)	0.05-0.17 (0.12)
10	BC-1	<LOD-1.1 (0.42)	ND	ND
11	BC-11	<LOD-1.5 (0.29)	ND	ND
12	TBMP	ND	<LOD-5.7 (3.6)	<LOD-3.6 (2.3)
13	2,6-DBP	ND	ND	<LOD-0.73 (0.13)
	POPs			
14	HCB	0.34-1.1 (0.57)	0.26-0.57 (0.38)	0.49-0.66 (0.56)
15	ΣPBDEs	(3.3)	(1.5)	(2.1)
16	ΣPCBs	(9.3)	(8.5)	(4.7)

Halogenated natural products

Q1 and other polyhalogenated 1´-methyl-1,2´-bipyrroles (PMBPs).
Q1 (Fig. 1a) was the most abundant HNP in all samples. Q1 concentrations decreased from site I (100 ng/g lipids) and site X (96 ng/g lipids) to A (43 ng/g lipids). All samples were at least three-fold higher contaminated with Q1 than with ΣPCBs (**Table 1**). Q1 had been detected in blubber of monk seals (*Monachus monachus*) from Mauretania (Northern Africa) at similar concentrations (6-76 ng/g lipids)[9] as in the present squids. Slightly higher Q1 concentrations (11-351 ng/g lipids) were determined in blubber of South African fur seals (*Arctocephalus pusillus pusillus*) from Namibia (Southern Africa).[10] However, Q1 concentrations in squid from sites I and X (~ 7,000 km more to the south) were higher than in monk seal blubber from Mauretania. This indicated that Q1 levels may increase from Northern to Southern Africa. In agreement with that, Q1 levels in environmental and food samples were usually more abundant in the southern than in the northern hemisphere.[11]
GC/ECNI-MS was used to detect further PMBPs in the sample. Based on the same response, the order was Q1 >> $BrCl_6$-MBPs > Br_2Cl_5-MBPs > Br_3Cl_4-MBPs in all samples from the three sites. Four $BrCl_6$-MBPs and four Br_2Cl_5-MBPs were detected in samples from each site. Similar PMBP isomer patterns were observed in oyster (*Crassostrea gigas*) from Lister Ley.[3] In addition, four Br_3Cl_4-MBPs were detected in samples from sites I and X, but the isomer pattern was different in all samples from site A. Differences between samples from the southern hemisphere (predominance of highly chlorinated PMBPs) and samples from North America (predominance of highly brominated PMBPs) had been observed before.[12,13] Based on the GC/ECNI-MS-SIM response of the most abundant isotopic peak of the molecular ion, $BrCl_6$-MBPs reached on average ~5-10% of Q1. Noteworthy, the natural producer of Q1 and other PMBPs are still unknown.

2,4,6-Tribromophenol (2,4,6-TBP).
Mean 2,4,6-TBP (Fig. 1f) concentrations in squid were 17 ng/g lipid mass (sites I and X) and 28 ng/g lipid mass at site A (**Table 1**). Accordingly, 2,4,6-TBP concentrations were highest in squid from site A which is different to Q1 which had higher concentrations in squid from sites I and X (see above). This suggests local sources for the individual HNPs so that their distribution is not as predictable as for anthropogenic POP.[1] Alga are known producers of 2,4,6-TBP.[14]

Polyhalogenated 1,1´-dimethyl-2,2´-bipyrroles (PDBPs).
GC/ECNI-MS-SIM screening for PDBPs with 1-6 halogen substituents (Cl + Br = 6) verified the presence of Br_4Cl_2-DBPs and Br_6-DBP in squid samples from all sites. In general, PDBP concentrations were low (mean concentrations of BC-10 ~1-2.0 ng/g lipids at sites I, X, and A). Interestingly, squid from site I not only featured BC-10 (Fig. 1b) but two further Br_4Cl_2-DBP isomers at lower abundance (**Fig. 3**, left). Unexpectedly, only BC-10 was detected at site A even though BC-10 was more abundant than at the other sites (**Fig. 3**, right). Moreover, Br_6-DBP reached only ~60% of Br_4Cl_2-DBP at site A, while Br_6-DBP was ~1.5 times more abundant than Br_4Cl_2-DBP at sites I and X.

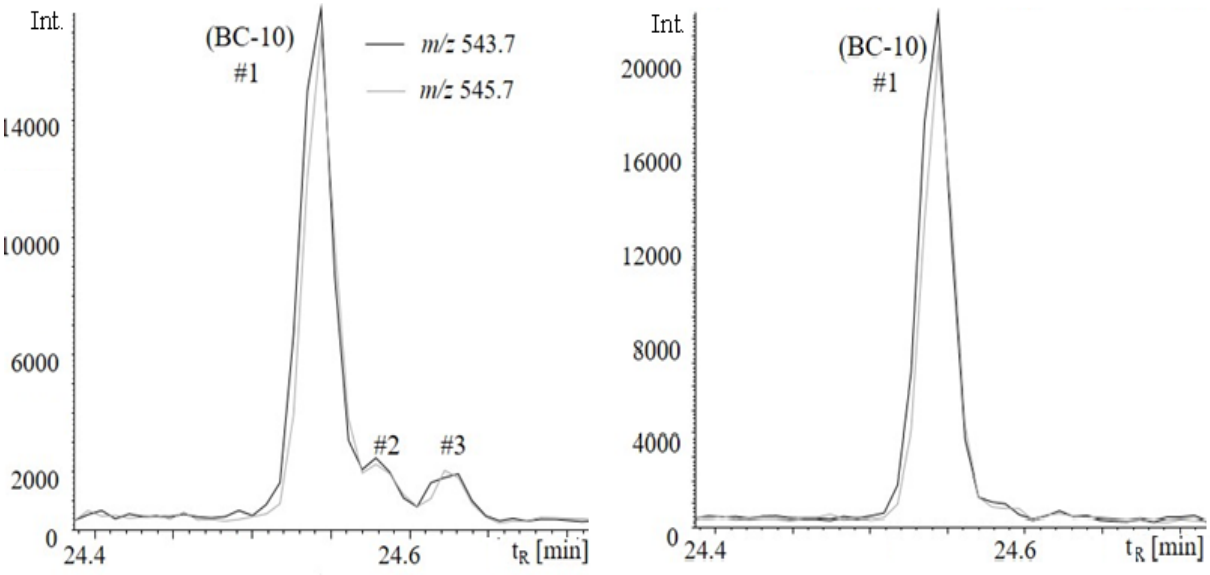

Figure 3. GC/ECNI-MS ion chromatograms of PDBPs in squid from the Indian Ocean (site I, left) and the Atlantic Ocean (site A, right).

Other differences between the sites had already been observed and discussed for Q1 and 2,4,6-TBP (see above). These variations in the Br_4Cl_2-DBP isomer pattern indicated variations in the natural production at different sites. Generally, Br_6-DBP is predominant in areas influenced by Atlantic Ocean, and Br_4Cl_2-DBP is predominant in areas influenced by the Pacific Ocean.[15] In our study, the distribution pattern of PDBPs congeners at sites I and X were consistent with above rules. Previous studies had reported that PDBPs appear to be primarily produced in the northern Pacific Ocean and is distributed worldwide by atmospheric and ocean current transport, while not excluding the influence of different marine organisms distributed in various locations.[15]

Other HNPs

2´-MeO-BDE 68 (BC-2, Fig. 1d), 6-MeO-BDE 47 (BC-3, Fig. 1e), 2,4-DBA and 2,4-DBP were frequently detected in squid from the three sites. Overall, BC-2 and BC-3 were the most abundant at site I, followed by site X and site A (**Table 1**). BC-2 and BC-3 may be synthesized mainly by sponges, algae and associated organisms, although they can also be generated by biotransformation of hydroxylated PBDEs.[1,16]

The mixed halogenated compound (MHC-1, Fig. 1c) was detected at low concentrations at each site, i.e. <1 ng/g lipids (**Table 1**). The presence of MHC-1 has been linked with the distribution of *Plocamium* sp.,[17] which is present in the ocean waters off South Africa. Finally, we detected tetrabromo-1-methylpyrrole (TBMP) with up to 3.6 ng/g lipids at site X (**Table 1**).

HNPs versus anthropogenic POPs.

The sum of anthropogenic contaminants (mainly PCB 153, PCB 138, PCB 153 and BDE 47) in squid was only 7.0% (site I), 4.9% (site X) and 6.1% (site A) of the HNPs. Hence, HNPs were one order of magnitude more relevant than anthropogenic contaminants, which was mostly due to the predominance of Q1. In all samples, Q1 was more abundant than sum PCBs, PBDEs, HCHs, HCB, and ΣDDT (which had surprisingly low concentrations in the squid samples). Most HNPs had their highest concentrations at site I (located in the Indian Ocean). One exception was 2,4,6-TBP, which had the highest concentrations at site A in the Atlantic Ocean. Different distribution of HNPs between the three sites at the South African suggest differences in HNPs producers, therefore confounding predictions of the occurrence and concentrations of HNPs in the marine environment.

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