NATURAL AND MAN-MADE ORGANOBROMINE COMPOUNDS IN EGGS AND LIVERS OF EUROPEAN SHAG FROM SKLINNA, CENTRAL NORWAY

Walter Vetter[1], Roland von der Recke[1], Dorte Herzke[2], and Torgeir Nygård[3]

[1] University of Hohenheim, Institute of Food Chemistry (170), Garbenstr. 28, D-70599 Stuttgart, Germany
[2] Norwegian Institute for Air Research (NILU), The Polar Environmental Centre, Hjalmar Johansens gate 14, NO-9296 Tromsø, Norway
[3] Norwegian Institute for Nature Research (NINA), Tungasletta 2, NO-7485 Trondheim, Norway

Introduction

Anthropogenic organobromine compounds (AOBs) belong to the class of halogenated pollutants that currently receive increased attention in the scientific literature. Mainly used in fire prevention, recent research has indicated that they possess similar adverse environmental properties as chlorinated compounds with related structure[1]. Because of ecotoxicological concerns along with previous accidents, the use of both PBBs and PBDEs has been restricted[2].

Halogenated natural products (HNPs) are widespread in nature[3]. Relatively new, however, is linking them with environmental issues, which means that the HNPs are detected in higher organisms that were not the natural sources but have accumulated the natural products[4]. The co-existence of both HNPs and anthropogenic organobromine pollutants (AOPs) has been documented[5-7].

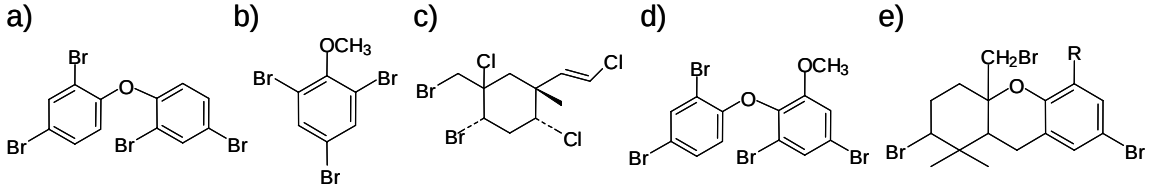

Figure 1: structures of (a) the anthropogenic BDE 47 (b) the mainly naturally-produced 2,4,6-tribromoanisole (TBA), (c) an isomeric structure of naturally produced MHC-1, (d) the HNP 3,5-dibromo-2-(2´,4´-dibromo)phenoxyanisole and (e) the substitution pattern of tribromo (R = H= and tetrabromo (R = Br) hexahydro-1*H*-xanthene derivatives (PBHDs) which are natural products as well.

In a previous study of eggs of Norwegian birds of prey, both AOPs and HNPs were determined in diverse species but the individual congener pattern was very varied[8]. In this study, we screened five sets of eggs, livers and chicks from the same nest of European shag *Phalacrocorax a. aristotelis* from the island of Sklinna, Central Norway (65º12'N 11º00'E). The samples chosen were the first egg in a nest as well as liver of medium-developed chicks (21 days). Time in or near the nest for the hatchlings is between 45-59 days[9]. The difference in the contamination sources is that eggs represent the uptake by exclusive transfer from the mother animal whereas the bird liver represents direct food-uptake and metabolism.

We were interested in studying the following points: (i) what is the variety of brominated compounds in the samples? (ii) are there any unknown compounds in the samples? (iii) is there a difference between the residue pattern of eggs and livers (iv) and finally, which of the two groups – HNPs or AOPs – is more important in the samples?

Material and Methods

Chemicals and standards. Organobromine reference standards were from Cambridge Isotope Laboratories, (BDE reference standard EO-4980, ^{13}C-labelled BDEs), Sigma-Aldrich (2,4,6-tribromoanisole, TBA), or synthesized/isolated/identified by some of us.

Sampling sites, samples, and ecology of shags. The sampling campaign was carried out at Sklinna, Central Norway, between May and July 2003. Sklinna, a protected major seabird colony, is an isolated group of islands about 200 km north of Trondheim in the Norwegian Sea where no direct pollution was expected except for transport/input via seawater and/or air. The nominate form of European shag is a medium-sized cormorant; it is a

marine bird throughout the whole year, and feeds on pelagic fish[9]. At Sklinna, the diet seems to be constituted mainly of 0-group and 1-year old gadoids. The egg taken was the first laid, distinguishable by its darker colour. The shag livers were taken from the smallest chick (by weight).

Sample preparation. Homogenised samples were extracted with cyclohexane/acetone (3:1, v/v), purified using gel permeation chromatography (GPC) and florisil column chromatography as described elsewhere[10]. $^{13}C_{12}$-labelled BDE 28, BDE 47, BDE 99, BDE 153, and BDE 183 were added prior to extraction. Octachloronaphthalene was added as recovery standard before quantification was carried out.

GC/ECNI-MS. Analyses were carried out with a 3800/1200 GC/MS system in combination with a 30 m x 0.25 mm i.d. x 0.25 µm d_f Factor Four® CP-Sil 8ms column (Varian, Darmstadt, Germany). Further parameters are reported elsewhere[11]. In the selected ion monitoring (SIM) mode, *m/z* 79, *m/z* 81, *m/z* 114, *m/z* 116, and *m/z* 158-161 were recorded throughout the run[6].

GC/EI-MS. PBBs and BDEs were analysed on an 8560 Mega (CE Instruments, Milan, Italy) GC in combination with an MD 800 (Finnigan, San Jose, CA, USA) MS employing parameters reported elsewhere[8].

Determination of organobromine compounds. BDE congeners were quantified by GC/EI-MS according to Herzke *et al*.[8,10]. The relevance of other organobromines was determined by GC/ECNI-MS. For this reason the ratio of isotope-labeled and native BDEs in individual sample extracts was determined by GC/EI-MS. Then, the relative contribution of the ^{13}C-labeled BDEs was subtracted from the GC/ECNI-MS response. Individual GC/ECNI-MS responses of BDEs were taken from the external BDE standard mix EO-4980. The quality of these determinations was checked with BDE 47 (^{13}C-standard subtracted) and BDE 100 (no ^{13}C standard used). All values except for shag egg D were in very good agreement. Given the good agreement obtained for the other samples, this procedure was also carried out for other BDEs in case of positive detection by GC/EI-MS. Following that, all organobromines were determined relative to the abundance of *m/z* 79 and *m/z* 81 of BDE 47.

Results and Discussion

Determination of brominated compounds in the samples investigated. In a first step, brominated compounds were determined by their correct ratio of *m/z* 79 and *m/z* 81 using GC/ECNI-MS-SIM. In a sample campaign that also included fish liver and mollusks, we detected 84 brominated compounds. The first eluting compound (#1) identified (a monobromophenol isomer) was detected at 5.5 min whereas the last one (#84) left the column shortly after BDE 207(~80 min). 10-23 and 21-44 organobromine compounds (OBCs) were detected in the shag liver and eggs, respectively. In this study we concentrated on the most remarkable compounds only (**Table 1**).

AOBs in the samples. BDE 47 (#53), BDE 100 (#57), and BDE 154 (#66) were the dominating congeners in shag eggs (**Table 1**). Significantly lower concentrated were BDE 28 (#43), BDE 49 (#51), and BDE 99 (#61) which were found at comparable concentrations. BDE 183 was not detected in any sample. This pattern is rather unique since BDE 153 did not play a significant role. By contrast, only BDE 47 was detected in all liver samples of hatchlings, whereas BDE 100 and BDE 99 were detected in one liver, respectively. The BDE pattern in liver was completely different to eggs. However, even in the eggs, BDEs did not play a significant role. Only in one egg sample BDE 100 belonged to the five most abundant organobromine compounds (**Table 1**). Based on both GC/EI-MS and GC/ECNI-MS analysis, less than 10 of the >80 peaks in the samples could be traced back to brominated flame retardants. Note that virtually all relevant BDEs described in technical products and environmental samples were available as reference standards. Thus, there was a need to screen the samples for HNPs as well.

Halogenated natural products (HNPs). Of the known HNPs, TBA (#13) (**2**) and MHC-1 (#34) were abundant in all samples (**Table 1**). The MHC-1/BDE 47 ratio was up to 17 fold more concentrated in shag (**Figure 2**). However, this ratio was usually lower in eggs than in liver of young shags. In addition, the HNPs BC-2 (2´-MeO-BDE 68, #54) and BC-3 (6-MeO-BDE 47, #56) (**4**) were low abundant in the eggs but BC-2 was abundant in mostz of the liver samples (**Table 1**). Peak #65 and peak #75 were identified as TriBHD and TetraBHD. Both PBHDs (**5**) were recently identified as bioaccumulating HNPs in commercial fish[12]**.** The presence of these naturally-produced compounds indicated that several other unknown peaks may also arise from HNPs. To get more information on the unknown peaks, a pattern evaluation was carried out.

Organobromine pattern analysis. The preceding subchapters showed that only ~1/3 of the compounds detected in shag could be traced back to known structures. The five most abundant compounds included TBA and MHC-1 but also unknown compounds such as #28 and #42 (Table 1). Comparable peak abundances of #28 and #42 in shag eggs pointed to similar sources and persistence of both compounds. However, both #28 and #42 were only detected in eggs but not in liver (**Figure 3a** and **3b**). In shag eggs, more compounds exceeded the concentrations of BDE 47 compared to shag liver. MHC-1 was a major compound in all samples and amounted for up to 17 fold the amount of BDE 47. Similarly to #28 and #42, TetraBHD and #72 were very abundant in all shag eggs but low or absent in the livers of hatchlings (**Table 1**). By contrast, #67 and #73 were more abundant in most liver samples (**Table 1**). The peak abundance of #73 was 1.2 – 21.1 fold that of BDE 47 in shag liver and 1±0.5 in shag eggs. #80 was only detected in two liver samples but accounted for 19 or 31% of the organobromine content, respectively (**Table 1**). Significantly higher variations in the abundance compared to other compounds were found for these unknown compounds. The distinct differences in the pollution pattern support the existence of local pollution sources. This could be differences in food choice or feeding-sites of the adults.

Table 1: Concentrations [ng/kg ww]* of polyhalogenated compounds in shag egg and liver from Sklinna

			shag egg					shag liver				
no.	**t_R [min]**	**name**	A	B	C	D	E	A	B	C	D	E
13	11.32	TBA	[a]	350	1410	**11400**	**450**	**990**	610	**770**	3320	**1090**
28	22.40		40	**1440**	**8030**	240	90					
34	24.56	MHC-1	**2200**	**3250**	**9260**	**11400**	**1580**	**1940**	**1090**	**1810**	**7550**	**780**
40	27.45		15	140	980							
42	28.26		46	**1610**	**6260**	220	68					
43	28.72	BDE-28	180	120	270		58					
51	34.34	BDE 49	190	55	170		32					
52	34.54		94	160	320	230	77	**290**	**740**	**350**	**7250**	**440**
53	35.31	BDE-47	1300	420	950	770	230	130	90	150	430	150
54	37.72	BC-2	65	53	90		23		630	180	**12840**	**470**
56	38.45	BC-3	210	190	230	360	59					
57	39.65	BDE-100	**1800**	580	1410	1150	310		54			
58	40.08		160	140	130		30				5990	
59	40.65		170		98				290		2560	180
60	40.84		380	480	1100	1930	120				2030	
61	41.00	BDE-99	240	28	110		21					200
64	41.95		**2110**	390	**2750**	**3260**	**320**					
65	42.58	TriBHD	190	120	250	490	66	150	98	150		
66	44.10	BDE-154	1170	260	550	470	150					
67	44.18		230	160	170		70	**450**	**1400**	**730**	6890	300
69	46.01	BDE-153			330							
72	46.96		**1380**	**1360**	1020	2450	**340**					
73	47.42		1040	720	1400	950	**330**	**420**	**1660**	**640**	**9220**	180
75	48.29	TetraBHD	**2000**	**900**	**3050**	**5570**	**320**	75	49			
79	64.20					**6650**						
80	67.00										**27740**	**1070**
81	73.90										2480	180
		SUM	16000	14200	43500	49600	4930	4700	8070	5700	88300	5670

* only compounds with at least one value exceeding 700 ng/kg ww or being a key BDE or one of the five most prominent compounds (printed bold) in the sample are shown, Concentrations were estimated using the GC/ECNI-MS response factor of BDE 47 except for other BDE congeners which were quantified using authentic reference standards.

Relative abundance of HNPs and AOBs as a tracer for the assignment of unknown compounds. As already indicated, the pattern of anthropogenic BDEs varied from different unknown OBCs. Whereas input of BDEs from external sources appears to be a key source in Central Norway, the unknown and potential HNPs seem to originate from local sources in shallow coastal marine environments. Organisms feeding in such habitats may thus receive significant amounts of HNP. Given such a gradient from the coast to the open sea, fish and also birds feeding at different distances from the coast will display different ratios of HNPs. This has to be considered when air samples are analyzed since the samplers are usually located directly at the coast. Other, well-known HNPs such as TBA and MHC-1 appear to be more evenly distributed along the Norwegian North(ern) Sea coastline which agrees with previous reports in air samples[13-14]. The known HNPs and the potential HNPs described in this study may be a potential problem for marine aquaculture, especially for mussels, which is widely distributed in Norway. A thorough screening for both polybrominated compounds should be carried out.

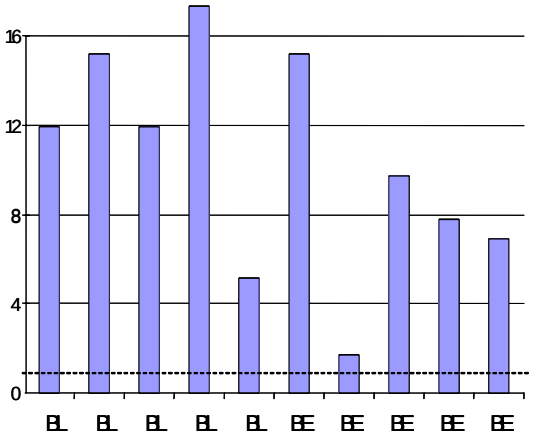

Figure 2: Ratio of MHC-1 to BDE 47 in shag liver (BL) and eggs (BE) from Sklinna

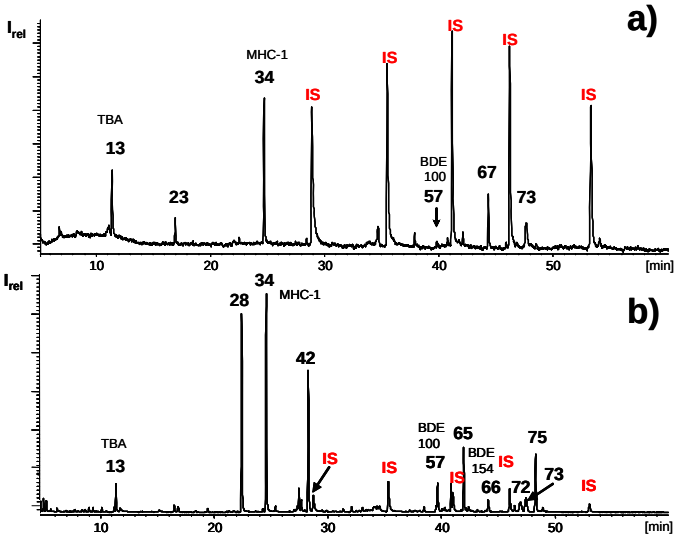

Figure 3: GC/ECNI-MS chromatograms of (a) shag liver and (b) shag egg from Sklinna. Internal standards are labeled IS, peak # assignment according to Table 1.

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