GAS CHROMATOGRAPHIC RETENTION DATA OF ENVIRONMENTALLY RELEVANT POLYBROMINATED COMPOUNDS

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Introduction

(Poly)brominated flame retardants (BFR) are currently in the focus of environmental chemists since several of these compounds extensively used in fire prevention were shown to be persistent, bioaccumulative, and frequently toxic[1-3]. The most-frequently BFRs in food and environmental samples are polybrominated diphenyl ethers (PBDEs, **Figure 1a**), hexabromocyclododecane (HBCD, **Figure 1d**), polybrominated biphenyls (PBBs, **Figure 1b**) and tetrabromobisphenol A (TBBPA). Except for TBBPA (which needs to be methylated prior to analysis), all other compounds are directly accessible to GC analysis. 1,2-*bis*(2,4,6-tribromophenoxy)ethane (BTBPE, **Figure 1c**), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE, **Figure 1e**) and allyl-2,4,6-tribromophenyl ether (ATE, **Figure 1f**) have also been described in environmental samples. In addition to the congeners present in the technical products, congeners formed by the reductive debromination can reach concentrations on one level with the congeners originally applied in fire prevention.

Moreover, several halogenated natural products (HNPs, **Figure 1g-l**) have been detected at high concentrations which frequently exceeded the BFR burden of marine organisms and food[4-5]. The HNPs include 2,4,6-tribromoanisole (TBA, **Figure 1k**), a dibromotrichloro monoterpene named MHC-1 (**Figure 1l**), 2,2´-dimethoxy-3,3´,5,5´-tetrabromobiphenyl (2,2´-diMeO-BB 80, BC-1, **Figure 1h**), two tetrabromophenoxy-anisoles -- 2´-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3) -- the methoxy-phenoxyanisole 2´,6-diMeO-BDE 68 (BC-11) (**Figure 1g**), several hexahalogenated 1,1´-dimethyl-2,2´-bipyrroles (HDBPs, **Figure 1j**), polybrominated hexahydroxanthene derivatives (**Figure 1i**), all of which are widespread and sometimes occurring at high amounts, i.e. in the high µg/kg or even the mg/kg range. Here, we measured more than 100 organobromines and discuss their importance and potential co-elutions using the classic 95% methyl-5% phenyl-polysiloxane GC capillary phase. About 40% of the compounds are not available as standards.

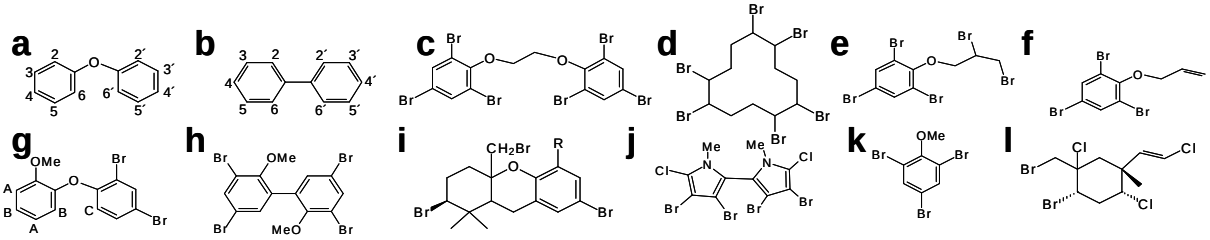

Figure 1: Structures of anthropogenic (top) and natural (bottom) compounds/compound classes studied

Material and methods

BDEs: A standard with 40 BDEs (EO-4980, CIL, Andover, USA) and congeners detected in technical products were studied. **PBBs:** identification of PBBs in technical mixtures and UV breakdown products was described elsewhere[6]. **Other BFRs**: HBCD was from Dr. Ehrenstorfer (Augsburg, Germany) and its metabolite pentabromocyclododecene (PBCDE) and the decomposition product "artifact 1" (A1) were determined as recently described[7]. ATE was from Merck (Darmstadt, Germany). DPTE and 2-bromoallyl-2,4,6-tribromophenyl ether (BATE) were synthesized as described elsewhere[8]. BTBPE was from Wellington (Guelph, Canada).

HNPs: 2´-MeO-BDE 68 (BC-2) was previously synthesized[9]. 6-MeO-BDE 47 (BC-3), 2,2´-diMeO-BB 80 (BC-1) and 2,6´-diMeO-BDE 68 (BC-11) were synthesized by Marsh *et al.*[10-11]. MHC-1 was isolated from a seaweed sample[12]. PBHDs -- 2,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene (TriBHD) and 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene (TetraBHD) -- were isolated from sponges[13]. 2,4-dibromophenol, 2,4,6-tribromophenol, and 2,4,6-tribromoanisole were from Sigma-Aldrich (Taufkirchen, Germany), 2,6-dibromophenol was from Lancaster (Frankfurt, Germany), and 2,4-dibromoanisole was from Alfa Aesar (Karlsruhe, Germany).

Internal standards (IS): a mixture of the following standards was added to the solutions measured for estblishing the retention times: ATE, BATE, DPTE, 2,6´-diMeO-BDE 68 (BC-11), and TetraBHD.

Gas chromatography in combination with electron capture negative ion mass spectrometry (GC/ECNI-MS). Analyses were performed with a 3800/1200 GC/MS system (Varian, Darmstadt, Germany). A DB-5-like HP-5ms column (30 m x 0.25 mm i.d. x 0.25 µm d_f) was used with the following GC oven program: after 2 min at 50 °C, the temperature was raised at 10 °C/min to 300 °C (hold time 38 min). Injections were performed in splitless mode (split opened after 2 min). He (Sauerstoffwerke, Friedrichshafen, Germany) was used as carrier gas with a constant flow of 1.2 mL/min. The ion source temperature was set at 150 °C. Methane (purity 99.995%, pressure ~8.5 Torr; Air Liquide, Bopfingen, Germany) was used as reagent gas. In the SIM mode, *m/z* 79, 81, 114, 116, 158-161 were recored throughout the run.

Results and Discussion

Measurements of standards. Except for BDEs and PBBs, all compounds were individually measured and the elution order was marked. BDEs were analyzed in form of a commercial fourty congener standard as well as the known technical products DE-71 and DE-79. Likewise technical hexabromobiphenyl and octabromobiphenyl and synthesized PBB 209 were analyzed as well as photochemically debrominated solutions of PBB 209 and technical octabromo diphenyl ether (TOBDE)[6]. After establishing of the elution orders and peak assignment, the standards were combined to five solutions including the five IS, respectively, and measured in subsequent runs. The IS chosen (see Material and Methods) covered a wide retention range (**Table 1**).

Anthropogenic brominated flame retardants.

PBDEs. Three technical PBDE products have been marketed. The technical pentabromodiphenyl ether (TPBDE) is composed of BDE 47 (~38%), BDE 99 (~49%, and BDE 100 (~13%) as the major congeners[14]. The technical octabromodiphenyl ether (TOBDE) mainly contains BDE 197 (~22%) and BDE 183 (~42%) whereas technical decabromodiphenyl ether is mainly composed of BDE 209 (>90%)[14]. Thirty-nine BDE congeners were identified as specific tri- to decaBDEs in the technical products[14]. BDEs elute according to the degree of bromination from nonpolar GC columns. Overlaps of congeners with different degree of bromination were only observed in two individual cases (the first eluting hexa-BDE 155 left the column prior to the last pentaBDE 105, **Table 1**).

Table 1: Relative retention times (t_R BDE 47 + BDE 180 = 1.0000[15]) of 110 organobromines studied

2,6-DBP	0.2221	2,2´-DMBB36	0.4228	PBB 136	0.4754	PBB 184	0.5156	BDE 181	0.5911
2,4-DBP	0.2300	DPTE	0.4299	PBCDE	0.4786	BDE 139	0.5187	PBB 201	0.5912
2,4-DBA	0.2530	BDE 75	0.4329	BDE 99	0.4785	BDE 140	0.5222	BDE 190	0.5962
2,4,6-TBA	0.2854	2,6´-DMBB36	0.4342	PBB 154	0.4820	PBB 179	0.5222	BDE 171	0.5977
2,4,6-TBP	0.2899	BDE 51	0.4346	BDE 116	0.4829	PBB 167	0.5245	PBB 197	0.6053
BDE 1	0.2944	BDE 49	0.4356	PBB 118	0.4830	HBCD	0.5268	PBB 189	0.6054
BDE 2	0.2972	BDE 71	0.4378	PBB 135	0.4845	PBB 176	0.5286	PBB 199	0.6116
BDE 3	0.3007	A1	0.4400	TriBHD	0.4862	TetraBHD	0.5304	PBB 203	0.6188
BDE 10	0.3151	BDE 47	0.4422	PBB 140	0.4908	BDE 138	0.5313	PBB 196	0.6225
ATE	0.3151	BDE 42/66	0.4475	PBB 149	0.4914	BDE 166	0.5337	BDE 201	0.6359
BDE 7	0.3374	BDE 77	0.4559	BDE 85	0.4935	PBB 178	0.5341	BDE 197	0.6442
BDE 8/11	0.3480	BC-2	0.4569	BDE 155	0.4953	PBB 187	0.5358	BDE 203	0.6563
BATE	0.3700	BC-1	0.4590	BDE 126	0.4960	PBB 174	0.5365	BDE 196	0.6644
MHC-1	0.3808	PBB 101	0.4590	PBB 133	0.4965	BDE 184	0.5504	PBB 194	0.6709
Q1	0.3848	BC-10	0.4598	BDE 105	0.4985	BDE 183	0.5578	BDE 205	0.6862
2,2´-DMBB11	0.3883	BC-3	0.4626	PBB 146	0.4986	PBB 172	0.5581	PBB 208	0.6867
2,6`-DMBB11	0.3935	BDE 102	0.4684	PBB 132	0.4991	PBB 180	0.5617	PBB 206	0.7783
BDE 17/25	0.3972	PBB 155	0.4697	PBB 153	0.5006	BDE 182	0.5618	BDE 208	0.8025
BDE 28/33	0.4027	BDE 100	0.4707	BDE 154	0.5009	BTBPE	0.5749	BDE 207	0.8181
BDE 35	0.4061	PBB 150	0.4716	PBB 188	0.5117	PBB 202	0.5810	BDE 206	0.8610
6,6´-DMB11	0.4071	BDE 119	0.4734	PBB 138	0.5120	BDE 180	0.5810	PBB 209	0.9219
BDE 37	0.4106	BC-11	0.4743	BDE 153	0.5124	PBB 170	0.5824	BDE 209	1.1614

In addition, the first hepta-BDE 184 eluted prior to the last eluting hexa-BDE 128 (both BDEs not available to us)[15]. More information on BDE elution orders can be extracted from the GC study of 126 BDE congeners provided by Korytar *et al*.[15].
Depending on matrix and site, dibromo- to decabromo-DEs may be detected in samples. However, in many cases -- including human milk[16] and marine mammals[17] -- BDE 47 and BDE 99 are the dominating congeners. In eggs of marine birds, BDE 153, BDE 154, BDE 183, and BDE 209 may be more abundant than BDE 47, BDE 99, or BDE 100[18-19]. BDE 209 may also be important in indoor samples (indicator for TDBDE) along with BDE 196 and BDE 197 (two key-congeners of TOBDE). Important low brominated congeners are BDE 17 and BDE 28 which co-elutes with BDE 33.

PBBs. Technical hexabromo biphenyl (THBB, key congeners: PBB 153 and PBB 180), technical octabromo biphenyl (TOBB, key congeners: PBB 183 and PBB 206), and technical decabromo biphenyl (TDBB, PBB 209) were marketed. THBB was mostly used in North America, but TOBB and TDBB played an important role in Europe. If contamination can be traced back to THBB, the residue pattern is dominated by PBB 153 along with low amounts of PBB 132, PBB 138, and PBB 149 (usually depleted compared to PBB 153 in THBB), whereas residues of PBB 180 the second most abundant congener in THBB is often below the retention limit[6]. PentaBBs are also playing a role, and in bird eggs, PBB 99 and PBB 101 were among the dominating isomers[20]. In contrast to BDEs, PBBs are not leaving the GC column according to the degree of bromination. For instance octaBBs can overlap with both hexa- and heptaBBs (**Table 1**)[20]. If pollution originated from TDBB, the PBB residue pattern is quite different. PBB 209 is readily degraded by sunlight, and the dominating congeners in fish and marine mammals are penta- and hexaBBs[6]. The most prominent congeners were PBB 153, PBB 154, and PBB 155[6]. Since PBB 155 is low abundant in THBB and absent in TOBB and TDBB, elevated amounts relative to PBB 153 are a direct proof of the debromination of higher brominated biphenyls; the same applies for PBB 154[6]. Other important hexaBBs in marine mammals and fish include PBB 149, PBB 132/146, PBB 136/148, and PBB 150[6]. In bird eggs from Europe several heptaBBs (PBB 188, PBB 178, and the co-eluting PBB 187/175) and pentaBBs were detected[20]. PBB levels are often one or two orders of magnitude lower than BDEs. Since the major BDEs in environmental samples are tetra- and pentabrominated and the major PBBs are hexabrominated, the dominating hexaBBs can reach or exceed the hexaBDE levels in environmental samples. Given the similiarity of BDEs and PBBs it is worth mentioning that PBBs eluted earlier than the corresponding BDEs. The difference in t_R increased with increasing degree of bromination (**Table 1**).

Further BFRs. When analyzed by GC, the three HBCD isomers (α-, β-, and γ-HBCD) cannot be separated[21]. HBCD which eluted ~0.25 min before BDE 138, and its potential breakdown products PBCDE (not separated from BDE 99) and A1 (~0.1 min before BDE 47) (**Table 1**) should be kept in mind as it may be abundant in GC/ECNI-MS-SIM chromatograms. A1 is likely a tetrabromo-degradation product of HBCD. BTBPE (**Figure 1c**) eluted between BDE 183 and BDE 180 (**Table 1**). DPTE and ATE (**Table 1**) differ only by the addition of Br_2 to the latter[8]. DPTE eluted slightly prior to BDE 75 from the GC column (**Table 1**). BATE, which is a metabolite of DPTE, eluted ~0.65 min before BDE 47 from DB-5 (**Table 1**).

Halogenated natural products.

Bromophenols and bromoanisoles. These early eluting HNPs are frequently detected seafood. In marine fish, 2,4,6-Tribromoanisole (TBA) is usually dominating representative of this substance class[22]. Given the rather low number of bromine substituents (1-3) and the simple backbone, these compounds are the first eluting compounds reported in this study (**Table 1**). Noteworthy as well, the simple bromophenols can be analyzed without derivatization, and the resulting peaks are reasonable sharp (**Table 1**).

Polybrominated phenoxyanisoles (methoxy-BDEs). The dominating tetrabromo phenoxyanisoles, 2´-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3), left GC column (i) in this order and (ii) between BDE 71 and BDE 100 (**Table 1**). Frequently, both isomers are coexisting in samples, but one might dominate significantly over the other. It appears that 2´-MeO-BDE 68 was more abundant in samples from Australia whereas 6-MeO-BDE 47 dominated in samples from the Baltic Sea (Europe)[23].

Dimethoxylated PBBs and BDEs. Both 2,6´-diMeO-BDE 68 (BC-11) and 2,2´-diMeO-BB 80 (BC-1, **Figure 1h**) have been identified in environmental samples[11,24]. Highest abundance was found in marine mammals from Australia and Japan. Retention times of further dibromo- and tribromo diMeO-BBs are also listed in **Table 1**.

Halogenated 1,1´-dimethyl-2,2´-bipyrroles (HDBPs). The hexahalogenated 1,1´-dimethyl 2,2´-bipyrroles are usually fully halogenated in the aromatic part but there might be either bromine/chlorine in the ratio 6/0, 5/1, 4/2, 3/3 (two isomers)[25]. The most important one HDBP in the environment is 4/2. If this congener (which elutes ~0.6 min before BDE 100, **Table 1**) is present, it is suggested to screen samples also for other HDBPs.

PBHDs. TriBHD eluted between BDE 116 and BDE 85 whereas TetraBHD left the column slightly before BDE 138 (**Table 1**). Noteworthy, the GC/ECNI-MS responses of the PBHDs were much lower compared to BDEs and PBBs[13,26]. A thorough investigation of fish oil dietary supplements showed that the two PBHDs were on average 9-fold more concentrated than all PBDEs together[26].

Co-eluting compounds of different substance classes. Due the many compounds studied, certain co-elutions were observed (**Table 1**). It was found that some PBBs detected in environmental samples co-eluted with BDEs (BDE 116/PBB 118; BDE 105/PBB 146; BDE 140/PBB 179; BDE 154/PBB 153[27-28]; BDE 183/PBB 172; BDE 182/PBB 180; BDE 180/PBB 202; BDE 181/PBB 201). Some may have been overlooked because low-brominated biphenyls were not available. Further relevant co-eluting compounds were ATE /BDE 10/lindane; BDE 51/2,6´-DMBB 36; BC-1/PBB 101; PBCDE/BDE 99, and HBCD/PBB 176 (**Table 1**).

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