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Gas chromatography retention data of environmentally relevant polybrominated compounds

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Abstract Polybrominated organic compounds are ubiquitous throughout the environment. This generic term comprises several classes of brominated flame retardants (e.g., polybrominated diphenyl ethers, polybrominated biphenyls, hexabromocyclododecane, dibromopropyltribromophenyl ether, 1,2-bis(2,4,6-tribromophenoxy)ethane) as well as a range of marine halogenated natural products (HNPs). Here we present gas chromatography retention times and elution orders (on DB-5) of 122 polybrominated compounds that may be found in food and environmental samples. Organobromine compounds in fish samples determined with gas chromatography interfaced to electron-capture negative ion mass spectrometry (GC/ECNI-MS) are discussed. The environmental relevance and important mass spectrometric features of the compounds are described as well. Our database aims to support the closer inspection and identification of peaks in gas chromatograms and to initiate dedicated screening for less frequently studied organobromines in samples.

Keywords Retention times · GC elution order · Polybrominated compounds · Polybrominated flame retardants · Naturally produced organobromines

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Introduction

(Poly)brominated flame retardants (BFRs, Fig. 1a–f) have been extensively used in fire prevention for decades. Several BFRs were shown to be persistent and bioaccumulative, and potentially hazardous [1, 2]. The unintended release of BFRs into the environment has been documented in many studies [1–7]. The most commonly detected group of BFRs in food and environmental samples are polybrominated diphenyl ethers (PBDEs, Fig. 1a) which exist in a theoretical variety of 209 congeners [1, 4–7]. Other BFRs frequently analyzed include 1,2,5,6,9,10-hexabromocyclododecane (HBCD, Fig. 1d) [8, 9], polybrominated biphenyls (PBBs, Fig. 1b), hexabromobenzene (HBB), as well as tetrabromobisphenol A (TBBPA) [1, 5]. Except for TBBPA (whose hydroxyl groups need to be derivatized prior to GC analysis), all other compounds are directly accessible to GC analysis. In addition, new or "forgotten" BFRs were repeatedly detected in environmental samples. For instance, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE, Fig. 1c) has been determined in air from electronics recycling plants [10, 11] and in bird eggs [12], whereas 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE, Fig. 1e) and allyl-2,4,6-tribromophenyl ether (ATE, Fig. 1f) were only scarcely studied but occasionally found with relatively high concentrations in the environment [13, 14]. However, it is not just the BFRs distributed via the application of the technical products that are of environmental concern; metabolites formed by reductive debromination or in some other way can reach concentrations on one level with the congeners originally applied in fire prevention. Thus, a series of additional PBDE and PBB congeners as well breakdown products of HBCD and DPTE are also potential pollutants of environmental samples. Even in recent publications the identity of several highly brominated diphenyl ethers could

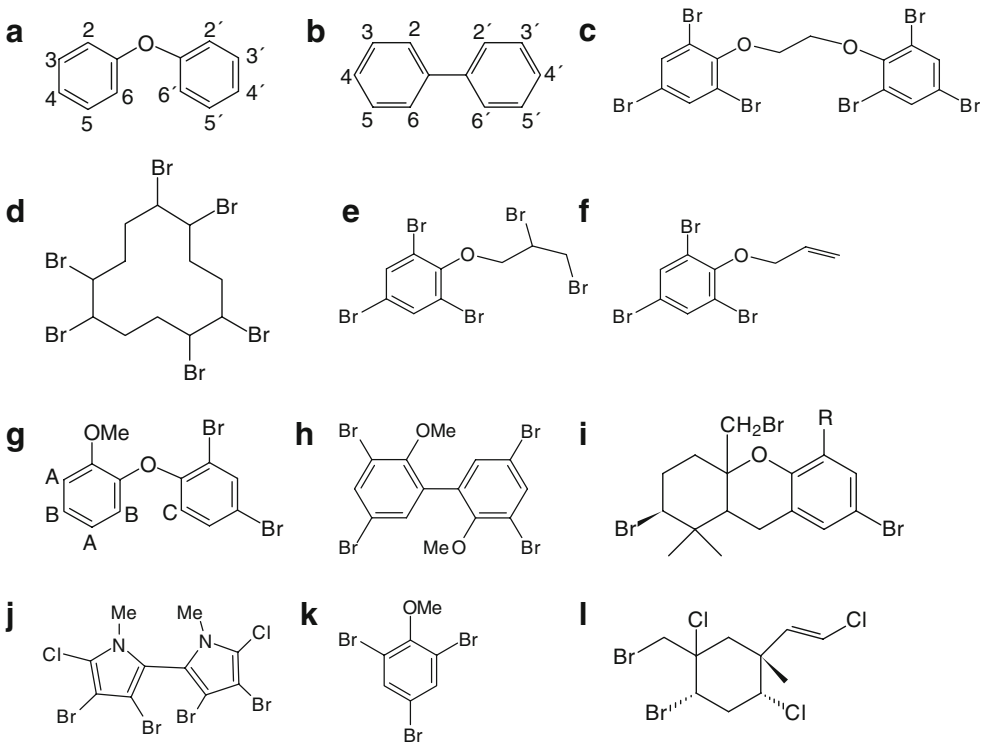

Fig. 1 Structures of brominated flame retardants (**a**–**f**) and halogenated natural products (HNPs) (**g**–**l**) discussed in this study. **a** Polybrominated diphenyl ethers and **b** polybrominated biphenyls exist in a theoretical variety of 209 congeners, respectively. The substitution patterns of the compounds studied are given in Table 1. **c** 1,2-Bis (2,4,6-tribromophenoxy)ethane, **d** 1,2,5,6,9,10-hexabromocyclododecane (stereochemistry not shown), **e** 2,3-dibromopropyl-2,4,6-tribromophenyl ether, and **f** allyl-2,4,6-tribromophenyl ether. The *last two rows* show HNPs: **g** the polybrominated phenoxyanisoles 2′-MeO-BDE 68 (where A = Br) and 6-MeO-BDE 47 (where B = Br) and the methoxy-phenoxyanisole 2′,6-diMeO-BDE 68 (where A = Br and C = OMe), **h** 2,2′-diMeO-BB 80, **i** two polybrominated hexahydroxanthene derivatives (R = H or Br), **j** hexahalogenated 1,1′-dimethyl-2,2′-bipyrroles (the most prominent congener is shown), **k** 2,4,6-tribromoanisole, and **l** the dibromotrichloro monoterpene MHC-1

not be solved despite ongoing efforts in the synthesis and distribution of individual PBDEs [15].

The situation is getting even more complex since several halogenated natural products (HNPs, Fig. 1g–l) have been detected at high concentrations which frequently exceeded the BFR burden of marine organisms and food [16, 17]. The HNPs include the 2,4,6-tribromoanisole (TBA, Fig. 1k) [18], a dibromotrichloro monoterpene named MHC-1 (Fig. 1l) [18], the 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB 80, BC-1, Fig. 1h) [19], two tetrabromophenoxyanisoles 2′-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3) [20–23], the tetrabrominated methoxy-phenoxyanisole 2′,6-diMeO-BDE 68 (BC-11) (Fig. 1g) [19, 24], several hexahalogenated 1,1′-dimethyl-2,2′-bipyrroles (HDBPs, Fig. 1j) [25, 26] and heptahalogenated 1′-methyl-1,2′-bipyrroles [27–31], polybrominated hexahydroxanthene derivatives (PBHDs, Fig. 1i) [32, 33], all of which are widespread and sometimes occurring in high amounts, i.e., in the high microgram per kilo or even the milligram per kilo range [16, 17].

Polybrominated compounds are often quantified using GC interfaced to electron-capture negative ion mass spectrometry (GC/ECNI-MS) [4, 34, 35]. Performed in the selected ion monitoring (SIM) mode, this method takes advantage of the high abundance of the bromide ion isotopes (*m/z* 79 and *m/z* 81) which are often the base peak in full scan mass spectra, thus providing a high sensitivity of detection [4, 34, 35]. Other techniques include GC with electron ionization mass spectrometry (GC/EI-MS) in the SIM mode. Usually the molecular ion is selected for quantification by low- or high-resolution mass spectrometry. In either case knowledge of the compounds that may exist in environmental and food samples is essential. While 126 of the 209 BDE congeners could be studied [36], only about 30 out of 209 PBBs can be purchased. Likewise, only a few HNPs are commercially available as reference standards. Thus, false interpretations due to the lack of reference standards or the co-elution of relevant pairs of organobromine compounds cannot be excluded. For this reason, we measured 122 organobromine compounds and discuss their importance and potential co-elutions on the classic 95% methyl-5% phenyl-polysiloxane (DB-5) stationary phase. About 40% of the compounds are not commercially available as standards.

Materials and methods

Standards and samples

The following compounds were analyzed as single standards (if available) and in mixtures with other compounds.

PBDEs The reference standard EO-4980 of 40 PBDEs (see below) was from Cambridge Isotope Laboratories (Andover, USA). Technical pentabromodiphenyl ether (TPBDE) product DE-71 and technical octabromodiphenyl ether (TOBDE) product DE-79 (Great Lakes Chemical Cooperation, Indianapolis, IN, USA) were analyzed as well. Additional PBDEs (BDE 51, BDE 102, BDE 139, BDE 171, BDE 180, BDE 182, BDE 184, BDE 196, BDE 197, BDE 201, BDE 203, BDE 205, BDE 206, BDE 207, BDE 208, BDE 209) were identified in technical products or obtained by transformation experiments.

PBBs Technical hexabromobiphenyl (THBB) and technical octabromobiphenyl (TOBB) were from ULTRA Scientific (North Kingstown, USA). Synthesis of PBB 209 and identification of PBB congeners in the technical mixtures were according to von der Recke and Vetter [37, 38]. In addition, debromination products and congeners identified in birds and mammals were included in this study [39, 40].

Other BFRs Technical 1,2,5,6,9,10-hexabromocyclododecane (HBCD) was from Dr. Ehrenstorfer (Augsburg, Germany) and its metabolite pentabromocyclododecene (PBCDE) and the decomposition product "artifact 1" was determined as recently described [41]. Artifact 1 was most likely identical to a cluster of tetrabromocyclododecadienes (TBCDD) determined in house dust [42]. 2,3-Dibromopropyl-2,4,6-tribromophenyl ether (DPTE) and 2-bromoallyl-2,4,6-tribromophenyl ether (BATE) were synthesized as described elsewhere [13]. Allyl-2,4,6-tribromophenyl ether (ATE) was from Merck (Darmstadt, Germany). 1,2-Bis (2,4,6-tribromophenoxy)ethane (BTBPE) was from Wellington (Guelph, Canada). Hexabromobenzene (HBB) was from Acros Organics (Geel, Belgium).

HNPs 2′-MeO-BDE 68 (BC-2) was synthesized in our research group [43]. 6-MeO-BDE 47 (BC-3), 2,2′-diMeO-BB 80 (BC-1), and 2,6′-diMeO-BDE 68 (BC-11) were synthesized as described by Marsh et al. [19, 44]. (1*R*,2*S*,4*R*,5*R*,1′*E*)-2-Bromo-1-bromomethyl-1,4-dichloro-5-(2′-chloroethenyl)-5-methylcyclohexane (MHC-1) was isolated from a seaweed sample [45]. The PBHDs 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) were isolated from sponges [32]. 2,4-Dibromophenol, 2,4,6-tribromophenol, and 2,4,6-tribromoanisole (TBA) 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 in order to establish the retention times: ATE, DPTE, MHC-1, and TriBHD.

Fish samples The fish sample (unknown species) from River Neckar was collected in 1994 close to Stuttgart (Southern Germany), fish (unknown species) from Lake Baikal was bought in 1996 on a local market close to Ulan Ude (Russia), and cod livers were taken from fish caught off Iceland in 1991. The samples were analyzed between 1994 and 1997. The sample cleanup included closed-vessel microwave-assisted extraction, gel-permeation chromatography, and adsorption chromatography on deactivated silica [46].

Gas chromatography in combination with electron-capture negative ion mass spectrometry (GC/ECNI-MS)

All analyses were performed with a 3800 GC connected to a 1200 GC/MS system (Varian, Darmstadt, Germany). A DB-5-like HP-5ms column (30 m×0.25-mm i.d. × 0.25-μm d_f) was used with the following GC oven program: after 2 min isothermal at 50 °C, the temperature was raised at 10 °C/min to 300 °C which was held for 38 min. The total run time was 65 min. Injections were performed in splitless mode (split opened after 2 min). He (purity 99.9990%; Sauerstoffwerke, Friedrichshafen, Germany) was used as carrier gas with a constant flow of 1.2 mL/min. The electron energy was set at 70 eV, and the ion source temperature at 150 °C. Methane (purity 99.995%, pressure ca. 8.5 Torr; Air Liquide, Bopfingen, Germany) was used as reagent gas. In the SIM mode, *m/z* 79 and *m/z* 81 ($[Br]^-$), *m/z* 114, *m/z* 116, and *m/z* 118 ($[BrCl]^-$), *m/z* 158 and *m/z* 160 ($[Br_2]^-$), as well as *m/z* 159 and *m/z* 161 ($[HBr_2]^-$) were recorded throughout the run. Full scan analyses covered the ranged *m/z* 30–800.

Results and discussion

Measurements of standards

Except for PBDEs and PBBs, all compounds were individually measured and the elution order was marked. PBDEs were analyzed in the form of a commercial forty-congener standard as well as the known technical products DE-71 and DE-79. Likewise technical hexabromobiphenyl (THBB) and octabromobiphenyl (TOBB) were analyzed

along with photochemically debrominated solutions of PBB 209 and technical octabromodiphenyl ether (TOBDE) [38]. After establishing the elution orders and peak assignment, standards were combined and measured in subsequent runs. The IS chosen (see Material and methods) covered a wide retention range (see below) and were chosen for qualitative purposes in order to establish elution orders. Note that these compounds might be present in food and environmental samples. Relative retention indices were calculated relative to the sum t_R of BDE 47 and BDE 183 following the suggestion of Korytár et al. [36] for PBDEs.

Polybrominated diphenyl ethers (PBDEs)

PBDEs exist in a variety of 209 congeners. Three technical products of different degree of bromination have been marketed. The technical pentabromodiphenyl ether (TPBDE) contains BDE 47 (ca. 38%), BDE 99 (ca. 49%), and BDE 100 (ca. 13%) as the major congeners [47]. The technical octabromodiphenyl ether (TOBDE) mainly contains BDE 197 (ca. 22%) and BDE 183 (ca. 42%), whereas technical decabromodiphenyl ether is dominated by BDE 209 (>90%) [47]. Thirty nine BDE congeners could be identified as specific tri- to deca-BDEs [47].

In many environmental and food samples, PBDEs are the dominating BFRs, but exceptions are known (see below). Between 1981 and 2000, BDE concentrations exponentially increased in the Canadian Arctic [48]. A similar trend until the 1990s was also observed in mussels from France, but thereafter the concentrations were stable or decreased [49]. In many samples—including human milk [50] and plasma [51], as well as marine mammals [52]—BDE 47 and BDE 99 are the dominating congeners. Eggs of marine birds contained a range of higher-brominated congeners (BDE 153, BDE 154, BDE 183, and BDE 209) that were partly more abundant than BDE 47, BDE 99, or BDE 100 [53, 54]. These seven PBDE congeners and BDE 197 were estimated to be those with the highest annual production rates [55].

BDE 209 may be one of the most prominent PBDEs in house dust and indoor air [56]. The molecular weight of BDE 209 is >900 Da and exceeds the high-mass range of certain mass spectrometers. Although the molecular ion can be detected, the most prominent ion suitable for quantification is *m*/*z* 487/489 of the $C_6Br_5O^-$ ion [57]. Moreover, BDE 209 and other high-brominated PBDEs are at least partly metabolized by reductive debromination. For instance, BDE 209 is partially biotransformed by fish to lower-brominated diphenyl ethers [58]. Transformation of highly brominated diphenyl ethers to tetra- to hexa-BDEs in environmental samples might partly explain the dominating role of these although the higher-brominated technical products have been used in much higher quantities.

The key BDE congeners in environmental and food samples are the following (with increasing retention time): BDE 17, BDE 28, BDE 49, (indicator for debromination) [48, 59]), BDE47 BDE 66 (indicator for debromination) [59], BDE 100, BDE 119 (indicator for degradation) [60], BDE 99, BDE 154, BDE 153, BDE 183, BDE 197, BDE 196, and BDE 209. These congeners are commercially available without exception. However, for a detailed understanding of the source inputs and weathering of PBDEs, even more PBDE congeners need to be studied and evaluated [48]. As a consequence, several groups of PBDE homologs need to be analyzed, depending on matrix and site.

PBDEs elute according to the degree of bromination from nonpolar GC columns (Table 1, Fig. 2). On DB-5, overlap of the homolog groups was only observed in two individual cases. In fact, only the first-eluting hexa-BDE 155 left the column prior to the last penta-BDE 105 (and together with the penta-BDE 126) (Fig. 2). In addition, Korytár et al. also found that the first hepta-BDE 184 eluted prior to the last-eluting hexa-BDE 128 (both PBDEs were not available to us) [36]. Moreover, only one co-elution of isomers was reported for TPBDE [61]. Owing to the availability of more individual BDE standards additional (potential) co-elutions were reported [36]. For instance, the pairs BDE 8/BDE 11, BDE 17/BDE 25 (Fig. 2) and BDE 28/BDE 33 co-eluted from DB-5 (Fig. 2). In addition, BDE 66 and BDE 42 (not available), BDE 119 and BDE 120 (not available), BDE 118 and BDE 97 (not available), BDE 126 and BDE 155 (partly separated in this study, Fig. 2), BDE 190 and BDE 173 (not available) may co-elute on DB-5 [36].

When, GC/ECNI-MS-SIM is used, it is recommended not only to monitor the bromide isotope ions (*m*/*z* 79 and *m*/*z* 81 but also the $[HBr_2]^-$) fragment ion (*m*/*z* 159 and the dominating *m*/*z* 161) formed by all BDE congeners except for non-*ortho* congeners and some mono- and di-BDEs (Fig. 3a,b) [62]. Screening for this fragment ion next to the bromide ion isotope is a good verifier of PBDEs (however, see below). In contrast, M^- is usually much less abundant than the bromide ion isotope. However, the molecular ion is abundant in GC/EI-MS. In GC/EI-MS, the most intense isotopic peaks of M^+ (Table 1) or the $[M-2Br]^+$ fragment ion are used for quantification (except for high-brominated diphenyl ethers) [17]. Table 1 also lists the monoisotopic peak of the molecular ion of PBDEs (and all other compounds) as well as the ion suggested for quantification by high-resolution mass spectrometry (rounded to unity or better still to the first decimal, the respective ions are recommended for quantification by GC/EI low-resolution mass spectrometry). In addition to the data set of our study, more information can be extracted from the GC study of 126 BDE congeners provided by Korytár et al. [36].

Table 1 GC/MS data of 122 organobromine compounds

No.	t_R (min)	RRI[a]	PBDEs	PBBs	Other compounds[b]	Chemical name	Molecular formula	Monoisotopic mass[c]	Most abundant isotope peak[d]	Remarks
1	11.983	0.2221			26-DBP	2,6-Dibromophenol	$C_6H_4Br_2O$	249.9	251.8608	HNP
2	12.409	0.2300			24-DBP	2,4-Dibromophenol	$C_6H_4Br_2O$	249.9	251.8608	HNP
3	13.650	0.2530			24-DBA	2,4-Dibromoanisole	$C_7H_6Br_2O$	263.9	265.8765	HNP
4	15.393	0.2854			246-TBA	2,4,6-Tribromoanisole	$C_7H_5Br_3O$	341.8	343.7870	HNP, BFR
5	15.640	0.2899			246-TBP	2,4,6-Tribromophenol	$C_6H_3Br_3O$	327.8	329.7714	HNP, BFR
6	15.880	0.2944	BDE 1			2-Bromodiphenyl ether	$C_{12}H_9BrO$	248.0	247.9837	
7	16.033	0.2972	BDE 2			3-Bromodiphenylether	$C_{12}H_9BrO$	248.0	247.9837	
8	16.221	0.3007	BDE 3			4-Bromodiphenylether	$C_{12}H_9BrO$	248.0	247.9837	
9	17.000	0.3151	BDE 10			2,6-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	325.9	327.8921	
10	17.000	0.3151			ATE	Allyl-2,4,6-tribromophenyl ether	$C_9H_7Br_3O$	367.8	369.8027	BFR
11	18.200	0.3374	BDE 7			2,4-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	325.9	327.8921	
12	18.771	0.3480	BDE 8/11			2,4′-/3,3′-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	325.9	327.8921	
13	19.001	0.3522	BDE 12/13			3,4-/3,4′-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	325.9	327.8921	
14	19.205	0.3560	BDE 15			4,4′-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	325.9	327.8921	
15	19.444	0.3604	BDE 30			2,4,6-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	403.8	405.8027	
16	19.960	0.3700			BATE	2-Bromoallyl-2,4,6-tribromophenyl ether	$C_9H_6Br_4O$	445.7	449.7111	Metabolite of DPTE
17	20.534	0.3807	BDE 32			2,4′,6-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	403.8	405.8027	
18	20.542	0.3808			MHC-1	See Fig. 1l	$C_{10}H_{13}Br_2Cl_3$	395.8	397.8429	HNP
19	20.757	0.3848			Q1	Heptachloro-1′-methyl-1,2′-bipyrrole	$C_9H_3N_2Cl_7$	383.8	385.8086	HNP (not brominated)
21	20.947	0.3883			2,2′-DMBB11	2,2′-Dimethoxy-3,3′-dibromobiphenyl	$C_{14}H_{12}Br_2O_2$	369.9	371.9184	HNP
22	21.227	0.3935			2,6′-DMBB11	2′,6′-Dimethoxy-3,3′-dibromobiphenyl	$C_{14}H_{12}Br_2O_2$	369.9	371.9184	HNP
24	21.428	0.3972	BDE 17/25			2,2′,4-/2,3′,4-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	403.8	405.8027	
25	21.725	0.4027	BDE 28/33			2,4,4′-/2′,3,4-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	403.8	405.8027	Frequently detected PBDE
26	21.904	0.4061	BDE 35			3,3′,4-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	403.8	405.8027	
27	21.959	0.4071			6,6′-DMBB11	6,6′-Dimethoxy-3,3′-dibromobiphenyl	$C_{14}H_{12}Br_2O_2$	369.9	371.9184	HNP
28	22.151	0.4106	BDE 37			3,4,4′-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	403.8	405.8027	
29	22.810	0.4228			2,2′-DMBB36	2,2′-Dimethoxy-3,3′,5-tribromobiphenyl	$C_{14}H_{11}Br_3O_2$	447.8	449.8289	HNP
30	23.189	0.4299			DPTE	See Fig. 1e	$C_9H_7Br_5O$	525.6	529.6373	BFR
31	23.335	0.4326			HBB	Hexabromobenzene	C_6Br_6	545,5	551.5039	BFR
32	23.351	0.4329	BDE 75			2,4,4′,6-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	

Table 1 (continued)

No.	t_R (min)	RRI[a]	PBDEs	PBBs	Other compounds[b]	Chemical name	Molecular formula	Monoisotopic mass[c]	Most abundant isotope peak[d]	Remarks
33	23.423	0.4342			2,6′-DMBB36	2,6′-Dimethoxy-3,3′,5-tribromobiphenyl	$C_{14}H_{11}Br_3O_2$	447.8	449.8289	HNP
34	23.442	0.4346	BDE 51			2,2′,4,6′-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	
35	23.496	0.4356	BDE 49			2,2′,4,5′-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	Indicator for PBDE debromination
36	23.615	0.4378	BDE 71			2,3′,4′,6-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	
37	23.736	0.4400			TBCDD	Tetrabromocyclododecadiene	$C_{12}H_{16}Br_4$	475.8	479.7945	BFR, metabolite of HBCD
38	23.853	0.4422	BDE 47			2,2′,4,4′-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	Major PBDE in biota
39	24.139	0.4475	BDE 42			2,2′,3,4′-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	
40	24.139	0.4475	BDE 66			2,3′,4,4′-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	
41	24.594	0.4559	BDE 77			3,3′,4,4′-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	481.7	485.7111	
42	24.648	0.4569			BC-2	4,6-Dibromo-2-(2′,4′-dibromo) phenoxyanisole	$C_{13}H_8Br_4O_2$	511.7	515.7217	HNP (6-MeO-BDE 68)
43	24.758	0.4590			BC-1	See Fig. 1h	$C_{14}H_{10}Br_4O_2$	525.7	529.7373	
44	24.762	0.4590		PBB 101		2,2′,4,5,5′-Pentabromobiphenyl	$C_{12}H_5Br_5$	543.6	547.6267	Major pentaBB in biota
45	24.801	0.4598			BC-10	See Fig. 1j	$C_{10}H_6Br_4Cl_2N_2$	539.7	543.6601	HNP
46	24.954	0.4626			BC-3	3,5-Dibromo-2-(2′,4′-dibromo) phenoxyanisole	$C_{13}H_8Br_4O_2$	511.7	515.7217	HNP
47	25.270	0.4684	BDE 102			2,2′,4,5,6′-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	
48	25.338	0.4697		PBB 155		2,2′,4,4′,6,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	Major metabolite of TDBB
49	25.394	0.4707	BDE 100			2,2′,4,4′,6-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	Frequently detected PBDE
50	25.440	0.4716		PBB 150		2,2′,3,4′,6,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
51	25.538	0.4734	BDE 119			2,3′,4,4′,6-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	Indicator for PBDE debromination
52	25.584	0.4743			BC-11	See Fig. 1g	$C_{14}H_{10}Br_4O_3$	541.7	545.7322	HNP (2,6′-diMeO-BDE 68)
53	25.644	0.4754		PBB 136		2,2′,3,3′,6,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
54	25.811	0.4785	BDE 99			2,2′,4,4′,5-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	Frequently detected PBDE
55	25.818	0.4786			PBCDE	Pentabromocyclododecene	$C_{12}H_{17}Br_5$	555.7	559.7206	BFR, metabolite of HBCD
56	26.002	0.4820		PBB 154		2,2′,4,5′,6,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	Major metabolite of TDBB

57	26.049	0.4829	BDE 116			2,3,4,5,6-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	
58	26.056	0.4830		PBB 118		2,3′,4,4′,5-Pentabromobiphenyl	$C_{12}H_5Br_5$	543.6	547.6267	
59	26.138	0.4845		PBB 135		2,2′,3,3′,5,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
60	26.228	0.4862			TriBHD	See Fig. 1i, R = H	$C_{16}H_{19}Br_3O$	463.9	465.8966	HNP
61	26.478	0.4908		PBB 140		2,2′,3,4,4′,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
62	26.507	0.4914		PBB 149		2,2′,3,4′,5′,6-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
63	26.619	0.4935	BDE 85			2,2′,3,4,4′-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	
64	26.716	0.4953	BDE 155			2,2′,4,4′,6,6′-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	637.5	643.5301	
65	26.756	0.4960	BDE 126			3,3′,4,4′,5-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	
66	26.785	0.4965		PBB 133		2,2′,3,3′,6,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
67	26.892	0.4985	BDE 105			2,3,3′,4,4′-Pentabromodiphenyl ether	$C_{12}H_5Br_5O$	559.6	563.6216	
68	26.895	0.4986		PBB 146		2,2′,3,4′,5,6′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
69	26.923	0.4991		PBB 132		2,2′,3,3′,4,6′-/2,2′,3,4′,5,5′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
70	27.006	0.5006		PBB 153		2,2′,4,4′,5,5′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	Major PBB in biota
71	27.019	0.5009	BDE 154			2,2′,4,4′,5,6′-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	637.5	643.5301	Frequently detected PBDE
72	27.602	0.5117		PBB 188		2,2′,3,4′,5,6,6′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.5	705.4457	
73	27.621	0.5120		PBB 138		2,2′,3,4,4′,5′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
74	27.640	0.5124	BDE 153			2,2′,4,4′,5,5′-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	637.5	643.5301	Frequently detected PBDE
75	27.815	0.5156		PBB 184		2,2′,3,4,4′,6,6′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.5	705.4457	
76	27.982	0.5187	BDE 139			2,2′,3,4,4′,6-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	637.5	643.5301	
77	28.168	0.5222	BDE 140			2,2′,3,4,4′,6′-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	637.5	643.5301	
78	28.172	0.5222		PBB 179		2,2′,3,3′,5,6,6′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.5	705.4457	

Table 1 (continued)

No.	t_R (min)	RRI[a]	PBDEs	PBBs	Other compounds[b]	Chemical name	Molecular formula	Monoisotopic mass[c]	Most abundant isotope peak[d]	Remarks
79	28.293	0.5245		PBB 167		2,3′,4,4′,5,5′-Hexabromobiphenyl	$C_{12}H_4Br_6$	621.5	627.5352	
80	28.416	0.5268			HBCD	1,2,5,6,9,10-Hexabromocyclododecane	$C_{12}H_{18}Br_6$	635.7	641.6447	BFR
81	28.516	0.5286		PBB 176		2,2′,3,3′,4,6,6′-Heptabromodiphenyl ether	$C_{12}H_3Br_7$	699.4	705.4457	
82	28.614	0.5304			TetraBHD	See Fig. 1i, R = Br	$C_{16}H_{18}Br_4O$	541.8	545.8050	HNP
83	28.662	0.5313	BDE 138			2,2′,3,4,4′,5′-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	637.5	643.5301	
84	28.811	0.5341		PBB 178		2,2′,3,3′,5,5′,6-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
85	28.904	0.5358		PBB 187		2,2′,3,4′,5,5′,6-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
86		0.0000		PBB 182		2,2′,3,4,4′,5,6-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
87	28.940	0.5365		PBB 174		2,2′,3,3′,4,5,6′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
88	29.691	0.5504	BDE 184			2,2′,3,4,4′,6,6′-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	715.4	721.4406	
89	30.091	0.5578	BDE 183			2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	715.4	721.4406	
90	30.107	0.5581		PBB 172		2,2′,3,3′,4,5,5′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
91	30.303	0.5617		PBB 180		2,2′,3,4,4′,5,5′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
92	30.304	0.5618	BDE 182			2,2′,3,4,4′,5,6′-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	715.4	721.4406	
93	31.014	0.5749			BTBPE	See Fig. 1c	$C_{14}H_8Br_6O_2$	681.6	687.5563	BFR
94	31.339	0.5810		PBB 202		2,2′,3,3′,5,5′,6,6′-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542	
95	31.341	0.5810	BDE 180			2,2′,3,4,4′,5,5′-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	715.4	721.4406	
96	31.416	0.5824		PBB 170		2,2′,3,3′,4,4′,5-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457	
97	31.887	0.5911	BDE 181			2,2′,3,4,4′,5,6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	715.4	721.4406	
98	31.892	0.5912		PBB 201		2,2′,3,3′,4,5′,6,6′-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542	
99	32.159	0.5962	BDE 190			2,3,3′,4,4′,5,6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	793.4	801.3491	

100	32.241	0.5977	BDE 171		2,2′,3,3′,4,4′,6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	793.4	801.3491
101	32.650	0.6053		PBB 197	2,2′,3,3′,4,4′,6,6′-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542
102	32.660	0.6054		PBB 189	2,3,3′,4,4′,5,5′-Heptabromobiphenyl	$C_{12}H_3Br_7$	699.4	705.4457
103	32.992	0.6116		PBB 199	2,2′,3,3′,4,5,5′,6′-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542
104	33.383	0.6188		PBB 203	2,2′,3,4,4′,5,5′,6-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542
105	33.579	0.6225		PBB 196	2,2′,3,3′,4,4′,5,6′-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542
106	34.303	0.6359	BDE 201		2,2′,3,3′,4,5′,6,6′-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	793.4	801.3491
107	34.751	0.6442	BDE 197		2,2′,3,3′,4,4′,6,6′-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	793.4	801.3491
108	35.406	0.6563	BDE 203		2,2′,3,4,4′,5,5′,6-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	793.4	801.3491
109	35.839	0.6644	BDE 196		2,2′,3,3′,4,4′,5,6′-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	793.4	801.3491
110	36.192	0.6709		PBB 194	2,2′,3,3′,4,4′,5,5′-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542
111	37.014	0.6862	BDE 205		2,3,3′,4,4′,5,5′,6-Octabromobiphenyl	$C_{12}H_2Br_8$	777.4	785.3542
112	37.043	0.6867		PBB 208	2,2′,3,3′,4,5,5′,6,6′-Nonobromobiphenyl	$C_{12}HBr_9$	855.3	863.2647
113	41.987	0.7783		PBB 206	2,2′,3,3′,4,4′,5,5′,6-Nonobromobiphenyl	$C_{12}HBr_9$	855.3	863.2647
114	43.291	0.8025	BDE 208		2,2′,3,3′,4,5,5′,6,6′-Nonobromodiphenyl ether	$C_{12}HBr_9O$	871.3	879.2596
115	44.129	0.8181	BDE 207		2,2′,3,3′,4,4′,5,6,6′-Nonobromodiphenyl ether	$C_{12}HBr_9O$	871.3	879.2596
116	46.448	0.8610	BDE 206		2,2′,3,3′,4,4′,5,5′,6-Nonobromodiphenyl ether	$C_{12}HBr_9O$	871.3	879.2596
117	49.731	0.9219		PBB 209	Decabromobiphenyl	$C_{12}Br_{10}$	933.2	943.1731
118	62.648	1.1614	BDE 209		Decabromodiphenyl ether	$C_{12}Br_{10}O$	949.2	959.1680

[a] Relative retention index: retention time relative to the sum of t_R (BDE 47+BDE 183)
[b] Abbreviations are explained in the text
[c] Mass of the monoisotopic peak of the molecular ion (may be rounded to unity)
[d] Exact mass of the most prominent isotope peak of the molecular ion (may be rounded to unity for low-resolution MS)

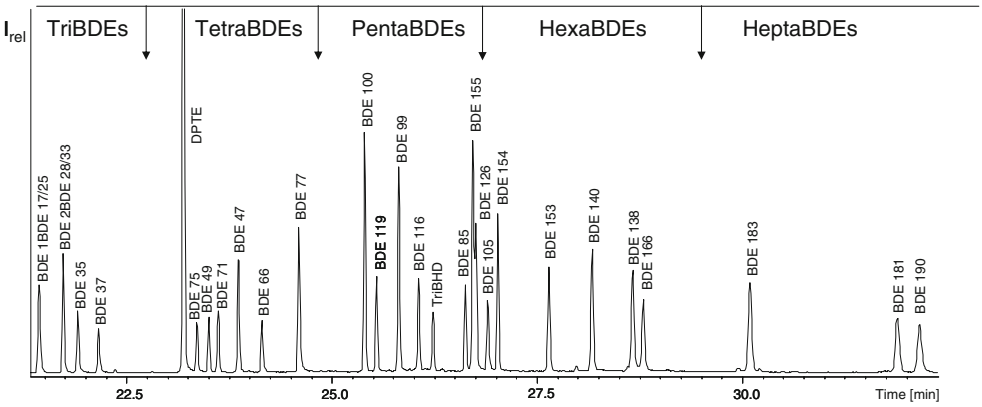

Fig. 2 GC/ECNI-MS-SIM chromatogram (*m/z* 79) of 29 tri- to hepta-BDEs along with the internal standards DPTE and TriBHD

Polybrominated biphenyls (PBBs)

Like the PBDEs, PBBs also exist in a variety of 209 congeners with one to ten bromine substituents on the biphenyl backbone. Three technical products THBB (key congeners: PBB 153 and PBB 180), TOBB (key congeners: PBB 183 and PBB 206), and TDBB (PBB 209) were marketed. While THBB was mostly used in North America (ca. 90% of the PBB production [63]), 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, low amounts of PBB 132 and PBB 149 (usually depleted compared with PBB 153 in THBB), whereas residues of PBB 180—the second most abundant congener in THBB—is often below the retention limit [39]. PentaBBs also play a role, and in bird eggs, PBB 99 followed by PBB 101 were among the dominating isomers [40]. Since not all congeners are commercially available, the most data exists on PBB 153 and PBB 101, the latter amounting for 10–30% of PBB 153 in lake trout from the Great Lakes [64].

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 hexa-BBs [39]. The most prominent congeners were PBB 153, PBB 154, and PBB 155 [39]. Since PBB 155 has a low abundance of THBB and no 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 [38]. Other important hexaBBs in marine mammals and fish include PBB 149, PBB 132/146, PBB 136/148, and PBB 150 [38]. Next to hexaBBs, several penta- and hepta-BBs were identified in marine biota which are not present in technical PBB products [39, 40]. In most samples—irrespective of whether contamination originated from TDBB, TOBB, or THBB—hexaBBs followed by pentaBBs dominate in marine mammals, fish, and bird eggs [39, 40]. However, bird eggs from Europe also contained several heptaBBs, the most prominent being PBB 188, PBB 178, and the co-eluting PBB 187/175 [40]. It is also noteworthy that many of the key PBBs mentioned above are neither present in the technical PBB products nor commercially available as standards.

Compared with PBDEs, PBB levels are often one or two orders of magnitude less concentrated in environmental samples. Since the major PBDEs in environmental samples are often tetra- and penta-bromo congeners and the major PBBs are hexabrominated, the dominating hexaBBs (PBB 153, PBB 154, and PBB 155) can reach or even exceed the hexaBDE levels in environmental samples.

In contrast to PBDEs (see above), PBBs do not leave the GC column according to their degree of bromination. For instance, early-eluting octaBBs can overlap with hexa- and hepta-BBs [40]. This is due to the strong impact of the number of Br atoms in the *ortho*-positions because such bulky substituents hinder the planarity of the phenyl rings which, in turn, weakens the interaction with the GC stationary phase. For this reason, PBBs with three and four

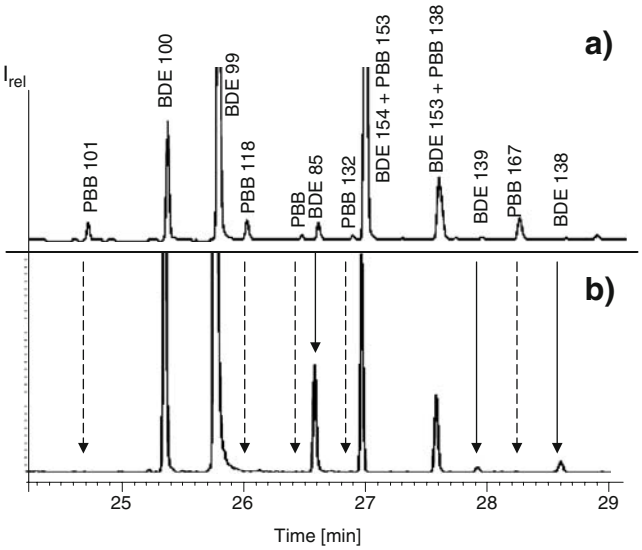

Fig. 3 GC/ECNI-MS-SIM analysis of a mixture of technical pentabromodiphenyl ether (DE-71) and technical hexabromobiphenyl (THBB): **a** *m/z* 79 gives response for PBDEs and PBBs, whereas **b** *m/z* 159 gives only response for PBDEs

bromine substituents in the *ortho*-positions elute comparably fast. Owing to this feature, i.e., the hindered rotation about the interannular phenyl–phenyl bond shared with non-symmetric substitution patterns on both rings, several PBBs are axially chiral [65]. Of the PBBs available to us, PBB 132/PBB 146, PBB 136/PBB 148, and PBB 187/PBB 175 had the same retention time (Table 1). Some co-elutions may have been overlooked because not all 209 congeners could be studied.

Using GC/ECNI-MS, the intensity of the molecular ion of PBBs is often <10% of the bromide ion isotopes. Fragment ions such as $[M-Br+H]^-$, $[M-2Br]^-$, $[M-3Br+H]^-$, and $[M-4Br]^-$ are usually even less abundant [38]. Low-mass fragment ions such as Br_2^- or HBr_2^- were not observed in the ECNI mass spectra of PBBs (Fig. 3a,b). Given the similarity of PBDEs and PBBs it is worth mentioning that (from hexabromo isomers on) PBBs eluted earlier than the corresponding PBDEs. The difference in t_R between PBDEs and PBBs increased with increasing degree of bromination (Table 1).

Further BFRs

Technical 1,2,5,6,9,10-hexabromocyclododecane (HBCD, Fig. 1d) is a mixture of three pairs of racemates (α-, β-, and γ-HBCD) [9, 66, 67]. While γ-HBCD is the dominating congener in technical hexabromocyclododecane, α-HBCD is often the dominating one in biota [9]. However, when analyzed by GC, the three isomers cannot be separated. Isomer-specific HBCD data have to be derived from LC/MS-MS measurements [8]. When analyzed by GC/MS, HBCD may decompose in the injector port, and thus an on-column injection has been recommended [68]. Nevertheless, under certain circumstances, HBCD can also be analyzed in splitless injectors with only minor decomposition [69]. Thus, the retention time of the HBCD peak (ca. 0.25 min before BDE 138) and its breakdown products PBCDE (co-eluting with BDE 99) and TBCDD (0.1 min before BDE 47) (Table 1) should be kept in mind as it may be abundant in GC/ECNI-MS-SIM chromatograms [69]. The HBCD peak in gas chromatograms is usually comparably broad because of the interconversion of isomers. PBCDE was detected at elevated concentrations in chicken eggs and selected fish [41, 69]. PBCDE is formed by the degradation of HBCD [41]. Recently, HBCD, four PBCDE isomers, and two tetrabromocyclododecadiene (TBCDD) isomers were determined in house dust [42]. The PBCDE and TBCDD pattern in the chromatograms shown by Abdullah et al. [42] was similar to the one observed by us. Thus, it is evident that the most prominent PBCDE isomer determined by Abdullah et al. is identical to the isomer listed in Table 1. Likewise, the peak originally labeled artifact 1 by Hiebl and Vetter [41] is most likely identical to the dominating TBCDD isomers described by Abdullah et al. [42]. In contrast to HBCD, the PBCDE peak(s) in gas chromatograms are very sharp. Depending on the status of the injector, the PBCDE peak (formed via decomposition of HBCD) can be smaller or larger. Thus, caution has to be undertaken to verify that the PBCDE and TBCDD are metabolites and not a decomposition product in GC [41]. Neither PBCDE nor TBCDD (artifact 1) are commercially available. For tracing them in gas chromatograms, HBCD should be injected and the injector temperature should be varied to achieve the decomposition products. Once the retention times are established (Table 1), the conditions should be altered such that PBCDE and TBCDD are minimized relative to HBCD. In general, samples containing HBCD should be analyzed with care because PBCDE and TBCDD may co-elute with the key PBDEs BDE 47 and BDE 99 (Table 1). Thus, it is recommended to quantify both HBCD as well as PBCDE and TBCDD by LC/MS [42].

DPTE (Fig. 1e) is formed by the addition of Br_2 to ATE (Fig. 1f), which itself was used as a BFR (Table 1) [13]. DPTE was marketed under the product name Bromkal 73–5 PE (Chemische Fabrik Kalk, Germany), and no other manufacturer has been reported [13]. Little is known about the production volume of DPTE. Commercial production may have started in the mid-1970s [13]. The previous lack of a commercially available reference standard might have hampered the identification of DPTE, but recently reference standards of DPTE and BATE have been introduced. The presence of DPTE and ATE in environmental samples has scarcely been reported, but in some of the few studies very high concentrations were reported. In addition, if present they are often found together. The first reported finding of DPTE in the environment was in 2003 [70]. Dedicated analysis proved that the compound initially described as UBC-1 [35] is actually DPTE [13]. DPTE eluted slightly prior to BDE 75 from the GC column (Fig. 2). High DPTE concentrations were reported in sewage sludge from Germany [71]. DPTE was shown to be selectively enriched in brain (ca. tenfold more than PBDEs) as was ATE [13]. A third DPTE-related compound is BATE, which is a metabolite of DPTE. DPTE elutes ca. 0.5 min before BDE 47 from DB-5 (Table 1). ATE may co-elute with lindane [13, 72], and BDE 10 (Table 1).

BTBPE (Fig. 1c) is used in the production of plastic materials that require high manufacturing temperatures and light-stability [73]. Production likely commenced around 1970 but comparably little data are available for this BFR. On the other hand it might become more frequently used because it may substitute TPBDE [74]. In recent years, BTBPE has repeatedly been described as a major contaminant in air of electronics recycling plants [10, 11]. The hexabrominated compound eluted between BDE 183 and

BDE 180 from the HP-5ms column (Table 1). Concentrations on one level with PBDEs have been reported in air from electronics recycling plants [10, 11] but also in birds [12]. When pyrolyzed, BTBPE is mainly converted into TBP (see below) and 2,4,6-tribromophenyl vinyl ether [75]. Other breakdown compounds of interest are polybrominated phenoxyphenols and dibenzo-*p*-dioxins [75]. It may be assumed that other transformation pathways may also lead to some of the pyrolysis products. BTBPE metabolism in the rat led to hydroxylated metabolites [73]. None of these breakdown products (except TBP) were available, but Balabanovich et al. presented a chromatogram along with GC/EI mass spectra which may be useful for a thorough screening of BTBPE-related compounds [75].

Hexabromobenzene (HBB) has been widely used as flame retardant in Japan [76]. With six bromine atoms on six carbons and no further elements, the bromine content of HBB is >85%. Recently, HBB concentrations in the range of minor PBDEs (BDE 28) were reported in birds from the Norwegian Arctic [77]. HBB left the column slightly before BDE 75 (ca. 0.5 min before BDE 47, Table 1).

Halogenated natural products

Bromophenols and bromoanisoles

These early-eluting HNPs are frequently detected in seafood and fish, and TBA (Fig. 1k) is often the dominating representative of this substance class [78]. TBA is most likely the metabolite of 2,4,6-tribromophenol (TBP) which is common in seafood and from which it is readily bioformed in the environment. As a consequence, marine fish usually contains TBA. Note that TBP has also been used as a BFR and that it is a breakdown product of other BFRs such as DPTE [13], BTBPE [75], and probably PBDEs (see above). Therefore, both anthropogenic and natural sources exist for TBP and TBA. In freshwater fish, TBP and TBA are likely of anthropogenic origin. Given the rather low number of bromine substituents (1–3) and the simple backbone, these compounds were the first-eluting compounds determined in this study (Table 1). It is also noteworthy that the simple bromophenols can be analyzed without derivatization, and the resulting peaks are reasonably sharp. The last-eluting TBP leaves the DB-5 column slightly before BDE 1 (Table 1).

Polybrominated phenoxyanisoles (methoxy-BDEs)

Tentatively described in marine mammals in 1997, MeO-BDEs are remarkable in that this class of compounds was identified as both HNPs as well as metabolites of BFRs. Natural sources could be unequivocally assigned to MeO-BDEs by (i) their isolation from algae [79] and sponges [24], (ii) their radiocarbon content [80], as well as their presence in pre-industrial archived samples [28]. On the other hand, some OH-BDEs are also metabolites of BFRs. For instance, hydroxylation of PBDEs to the corresponding OH-BDEs has been documented to occur by biotic and abiotic pathways [81, 82]. These polar organohalogens should be derivatized prior to GC analysis. Biomethylation converts OH-BDEs into MeO-BDEs which often coexist with the corresponding OH-BDEs [83]. There are two remarkable features which may be useful in differentiating naturally produced from anthropogenic MeO-BDEs. First, all environmentally relevant natural phenoxyphenols and phenoxyanisoles bear the methoxy group in the *ortho*-position (relative to the diphenyl ether backbone), whereas hydroxylation/methoxylation of PBDEs and pyrolysis of BTPBE may occur in *ortho*-, *meta*-, and *para*-positions [75, 82]. The *ortho*-substituted MeO-BDEs can be distinguished from those with the methoxy group in *meta*- or *para*-positions via GC/EI-MS [84, 85]. Second, two natural tetrabromophenoxyanisoles (also known as MeO-tetraBDEs) dominate in biota, i.e., 2′-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3). These two isomers leave GC columns in this order and between BDE 71 and BDE 100 (Table 1). Very often, both isomers coexist in samples, but one of them might dominate significantly over the other. It appears that 2′-MeO-BDE 68 (BC-2) was more abundant in samples from Australia (sponge-derived), whereas 6-MeO-BDE 47 (BC-3) dominated in European samples (most likely algae-derived [79]). MeO-triBDEs can be detected by GC/ECNI-MS tracing for *m/z* 159/161 which is only found for MeO-BDEs and PBDEs [62].

Dimethoxylated PBBs and PBDEs

Both a tetrabrominated dimethoxydiphenyl ether (2,6′-diMeO-BDE 68 (BC-11)) and a dimethoxybiphenyl (2,2′-diMeO-BB 80 (BC-1)) have been identified in environmental samples [19, 24]. Highest abundance was found in marine mammals from Australia and Japan. Recently, three minor diMeO-BBs were detected in Australian dolphins and it was assumed that these compounds may be metabolites of the parent compound 2,2′-diMeO-BB 80 [86]. The retention times of all relevant diMeO-BDEs and diMeO-BBs are listed in Table 1.

Halogenated dimethylbipyrroles (HDBPs) and methylbipyrroles (HMBPs)

HDBPs were described by Tittlemier et al. in Canadian marine birds [25]. Although the natural producer has not yet been identified, the natural source for HDBPs is no longer disputed. These hexahalogenated 1,1′-dimethyl-2,2′-bipyrroles are usually fully halogenated in the aromatic

parts but there might be bromine/chlorine present in the ratio 6:0, 5:1, 4:2, 3:3 (two isomers) [25]. The most important HDBP in the environment is the 5,5′-dichloro-3,3′,4,4′-tetrabromo-substituted derivative (Fig. 1j) which was structurally identified by Gribble et al. [87]. If this congener (which elutes ca. 0.6 min before BDE 100, Table 1) is present, it is suggested to screen samples for further HDBP congeners which were not available to us as standards. These compounds are most prominent in marine seabirds [25], but they have also been detected in marine mammals [31]. Although the highest concentrations have been found in the Northern Hemisphere, HDBPs have also been detected in samples from Japan and Australia [24, 88].

HMBPs are congeners of the (non-brominated) hepta-chloro-1′-methyl-1,2′-bipyrrole (Q1) (Table 1) [16, 23]. Following the first detection of HMBPs by Teuten et al. in 2006, more than thirty representatives of this substance class ranging from $BrCl_6$-congeners to the Br_7-MBP have been detected in environmental samples [27–31]. Unfortunately, groups of homologs elute in a very narrow retention range which cannot be fully resolved under typical GC conditions (see Materials and methods). Standards were not available to us, but retention times $BrCl_6$-MBPs (t_R 21.1–21.4 min) and Br_2Cl_5-MBPs (t_R 22.0–22.3 min) could be determined based on the previous determination in marine mammals [30].

Polybrominated hexahydroxanthene derivatives (PBHDs)

These compounds were only recently detected in fish from the Mediterranean Sea [32, 78]. One natural producer proved to be sponges [32]. TriBHD eluted between BDE 116 and BDE 85 (Fig. 2), whereas TetraBHD left the column slightly before BDE 138 (Table 1). It is noteworthy that the GC/ECNI-MS responses of the PBHDs were much lower compared with PBDEs and PBBs [32, 33]. A thorough investigation of fish oil dietary supplements showed that the two PBHDs were on average ninefold more concentrated than all PBDEs together [33].

Mixed halogenated compound 1 (MHC-1)

Discovered in marine fish in 2001 [18], MHC-1 (Fig. 1l) remained the only relevant HNP without heteroatoms except for halogens. The compound may co-elute with (non-brominated) *trans*-chlordane on DB-5. MHC-1 can be readily identified by GC/ECNI-MS by screening of the $[BrCl]^-$ (*m*/*z* 114, *m*/*z* 116) and $[Br_2]^-$ (*m*/*z* 158, *m*/*z* 160) fragment ions [18].

Co-elutions

Due the many compounds studied, several co-elutions were observed (Table 1). However, co-elution of different PBDE congeners or PBB congeners was rather scarce (see above). These problems are considered to be of minor importance only, since the compounds or co-eluting substances are of little environmental relevance. More relevant appear to be co-elutions between organobromine compounds from different substance classes. Co-elutions between PBBs and PBDEs were observed for the pairs BDE 116/PBB 118; BDE 105/PBB 146; BDE 140/PBB 179; BDE 154/PBB

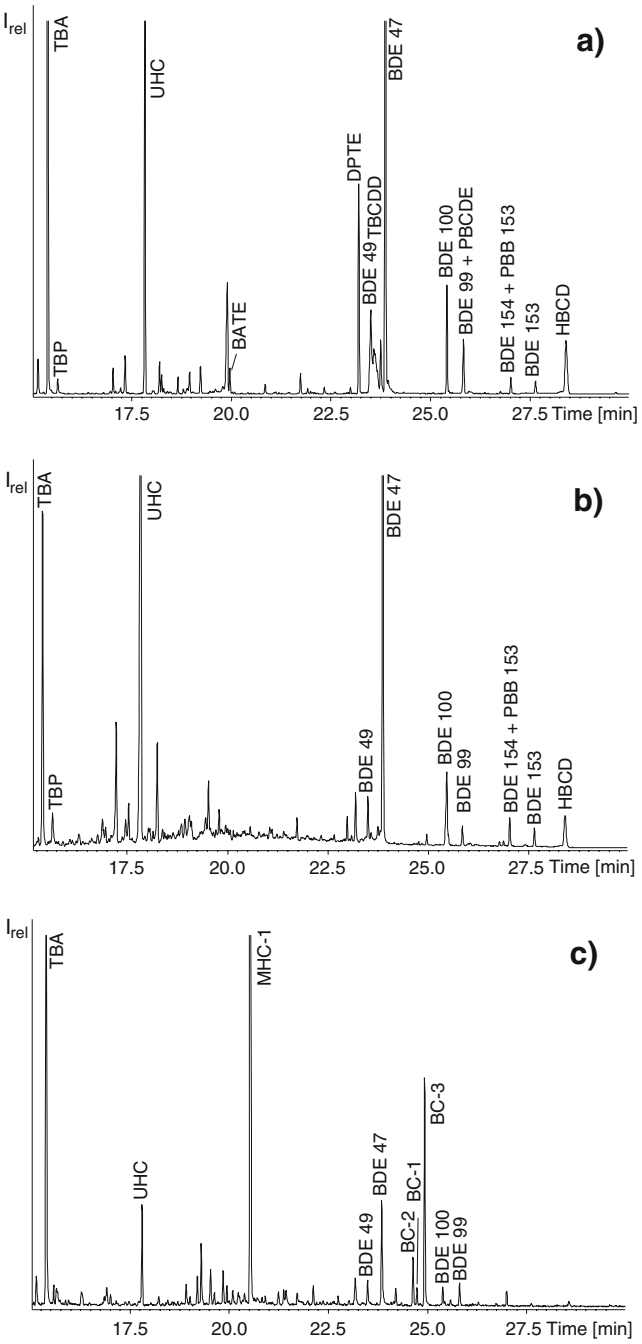

Fig. 4 GC/ECNI-MS-SIM chromatogram (*m*/*z* 79) of purified fish extracts from **a** the River Neckar (Southern Germany), **b** Lake Baikal, and **c** Iceland

153; BDE 183/PBB 172; BDE 182/PBB 180; BDE 180/PBB 202; BDE 181/PBB 201 (Table 1). The most important of these co-elutions is surely that of the two key congeners PBB 153 and BDE 154 (Fig. 3) [89, 90]. As a consequence, these two congeners could not be determined individually [90]. Moreover, it can be assumed that some of the data published on BDE 154 were falsified by the co-elution with PBB 153 if GC/ECNI-MS-SIM based on bromine ion isotopes was used for quantification. For verification of BDE 154 and other PBDEs, we suggest using *m/z* 159 and *m/z* 161 which is almost as abundant as *m/z* 79/81 but only formed from PBDEs and not from PBBs (Fig. 3b). The same problem exists in the case of BDE 153 and PBB 138 (Fig. 3). However, PBB 138 is of low environmental relevance so that this co-elution is less serious.

Further relevant co-eluting compounds were ATE/BDE 10/lindane. Although the last of these is not brominated, its retention time is known to virtually all environmental analysts, so that this information can be used for dedicated identification of ATE. Further important co-eluting pairs were BC-1/PBB 101 and PBCDE/BDE 99 (Table 1). The second is important because presence of PBDEs and HBCD in samples might lead to PBCDE being overlooked and/or concentrations of BDE 99 being overestimated. BDE 51/2, 6′-DMBB 36 and HBCD/PBB 176 also had the same retention time (Table 1).

Co-elution of the HNP MHC-1 with BDE 32 was observed. BDE 32 is only a very minor BDE, while abundance of MHC-1 may be varied. MHC-1 can be easily identified by screening for *m/z* 114/116 ($BrCl^-$), an ion not found on BDE 32. In this case, MHC-1 should not be quantified by the bromide ion without checking that *m/z* 114/116 is in the correct ratio. BDE 32 may be verified by *m/z* 159/161 ($[HBr_2]^-$) which is only found for BDE 32, whereas *m/z* 158/160 (Br_2^-) is found only for MHC-1. Finally, 2′-MeO-BDE 68 (BC-2) was not fully resolved from BDE 77.

Analysis of fish samples

Fish samples from different habitats were selected to demonstrate remarkable differences in their organobromine patterns. Fish from the River Neckar (Southern Germany), which has no access to the Oceans, only accumulated BFRs (Fig. 4a). Of the known compounds, BDE 47 was the dominating PBDE congener, but BDE 100 and BDE 99 were detected as well. TBA (most likely of anthropogenic origin) was identified as was DPTE and HBCD along with their metabolites BATE or PBCDE/TBCDD. An abundant peak was detected at 17.7 min, i.e., in the range where no compound of the standards studied eluted (Table 1). The GC/ECNI-MS mass spectrum of the unknown halogenated compound (UHC) gave response to both Br and Cl. That Br and Cl were found in the same compound (and did not originate from one organobromine compound co-eluting with one organochlorine compound) was verified by the detection of an abundant $[BrCl]^-$ fragment ion. A low-abundance ion cluster at *m/z* 390 (ca. 2% of the base ion) which appeared to be the molecular ion of UHC tentatively corresponded with the presence of three bromines and one chlorine. A plausible molecular formula could be $C_8H_6Br_3ClO$. Selective enrichment of UHC with the goal of structure determination has to be carried out in the future. Fish from Lake Baikal contained PBDEs (dominated by BDE 47), TBA, and HBCD. While DPTE was not detected, this fish sample also contained UHC (Fig. 4b). In contrast to these freshwater species, marine fish may also contain a range of HNPs. The GC/ECNI-MS organobromine pattern of cod livers from Iceland was dominated by the HNP MHC-1 (Fig. 4c). TBA and BC-3 were also more prominent than BDE 47 which again was the most abundant PBDE congener. In addition to PBDEs, this fish sample also contained UHC as well as the HNPs BC-1 and BC-2 (Fig. 4c). The peak at 27 min represented the co-eluting pair PBB 153 and BDE 154. Owing to the low abundance of *m/z* 161 (which identifies PBDEs), the bulk of the peak must originate from PBB 153 which has been previously detected in marine mammals from Iceland [39]. These samples also contained PBB 155 which co-eluted with BDE 100. Thus, the peak of BDE 100 also contained traces of PBB 155 (Fig. 4c). These examples illustrate that brominated compounds are widely distributed in food and environmental samples. The present database with retention times of known BFRs and HNPs may aid the comprehensive determination of known as well as the identification of unknown or less frequently detected organobromine compounds.

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