

# BROMOPHENOLS - SCARCELY DESCRIBED UV-TRANSFORMATION PRODUCTS OF POLYBROMINATED DIPHENYL ETHERS

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## Introduction

Bromophenols (BPs) were detected in various environmental compartments and foodstuffs. For instance, 2,4,6-triBP has been used as a reactive flame retardant and for wood preservation<sup>1</sup>. In addition to these anthropogenic sources, marine algae<sup>2</sup> and sponges are also producing bromophenols as secondary metabolites. The occurrence of BPs in seafood has even been linked with characteristic seafood flavors<sup>3</sup>. Naturally produced BPs were detected in passive water samplers from the Great Barrier Reef (Australia)<sup>4</sup>, mussels and seal blubber<sup>5</sup> and Norwegian air<sup>6</sup>. Additionally, bromophenols were identified as human metabolites of polybrominated diphenyl ethers (PBDEs)<sup>7</sup> and as transformation products of brominated flame retardants (BFRs) like 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) and BDE-209<sup>8,9</sup>. In the case of BDE-209 phenolic transformation products could only be detected after selective enrichment and derivatization.

In this study we aimed to investigate if and to what extent other PBDEs than BDE-209 could form bromophenols. For this reason we UV-irradiated different PBDE congeners in organic solvents and studied the formation of di- to pentaBPs.

## Materials and methods

**Gas chromatography coupled to electron capture negative ion mass spectrometry (GC/ECNI-MS).** An Agilent 7890/5975c GC/MS system was equipped with an HP-5MS UI column (30 m × 0.25 mm i.d., 0.25 μm d<sub>f</sub>, Agilent, Waldbronn, Germany). The injector (CIS-4, Gerstel, Mülheim, Germany) temperature was ramped from 80 °C (held for 0.01 min) at 500 °C/min to 260 °C until the end of the run. The transfer line, ion source and quadrupole temperatures were set at 280 °C, 150 °C and 150 °C respectively. The oven temperature started at 50 °C (held for 1 min) and then was raised at 10 °C/min to 300 °C (held for 14 min). Helium (5.0, Westfalen, Münster, Germany) was used as carrier gas at 1.2 mL/min. Methane (5.5, Air Liquide, Bopfinger, Germany) was used as the moderating gas. The ionization was performed with 175 eV and 150 μA. The following ions were recorded in the selected ion monitoring mode: from 8-16 min: *m/z* 79, 81 (for [Br]<sup>-</sup>), 172, 174 (for [M]<sup>-</sup> of monoBPs), 249.9, 251.9, 253.9 (for [M]<sup>-</sup> of diBPs), 327.8, 329.8, 331.8 (for [M]<sup>-</sup> of triBPs), from 16-22.5 min: *m/z* 79, 81 (for [Br]<sup>-</sup>), 329.8, 331.8 ((for [M]<sup>-</sup> of triBPs and [M-HBr]<sup>-</sup> of tetraBPs), 409.7, 411.7 (for [M]<sup>-</sup> of tetraBPs and [M-HBr]<sup>-</sup> of pentaBP) and 485.6, 487.6, 489.6 (for [M]<sup>-</sup> of pentaBP) and from 22.5-40 min *m/z* 79, 81 (for [Br]<sup>-</sup>), 159, 161 (for [HBr<sub>2</sub>]<sup>-</sup>), 329.8, 331.8 (for [M-HBr]<sup>-</sup> of tetraBPs and [M-Br<sub>2</sub>]<sup>-</sup> of pentaBP), 407.7, 409.7 (for [M]<sup>-</sup> of tetraBPs and M-HBr]<sup>-</sup> of pentaBP) and 434.7, 436.7 (for [M-Br]<sup>-</sup>) of 6-MeO-BDE-47). For determination of deuterated BPs formed by the irradiation of PBDEs in *tetradepro*-methanol (methanol-*d*<sub>4</sub>) the following ions were recorded: from 8-14 min: *m/z* 79, 81 (for [Br]<sup>-</sup>) and 247.9, 248.9, 249.9, 250.9, 251.9, 252.9, 253.9, 254.9, 255.9, 256.9, 257.9, 258.9 and 259.9 (for the [M]<sup>-</sup>-cluster of diBPs), as well as from 14-40 min: *m/z* 79, 81 (for [Br]<sup>-</sup>) and 325.8, 326.8, 327.8, 328.8, 329.8, 330.8, 331.8, 332.8, 333.8, 334.8, 335.8, 336.8 and 337.8 (for the [M]<sup>-</sup>-cluster of triBPs).

Quantitation of diBPs when no reference standard was available was performed by the average GC/ECNI-MS response of diBPs, while all tri- to pentaBPs were quantified with the response of 2,4,6-triBP. Retention time assignment of free bromophenols was based on the standards of 2,4-diBP, 2,6-diBP, 3,5-diBP and 2,4,6-triBP and according to Cristale et al.<sup>10</sup>.

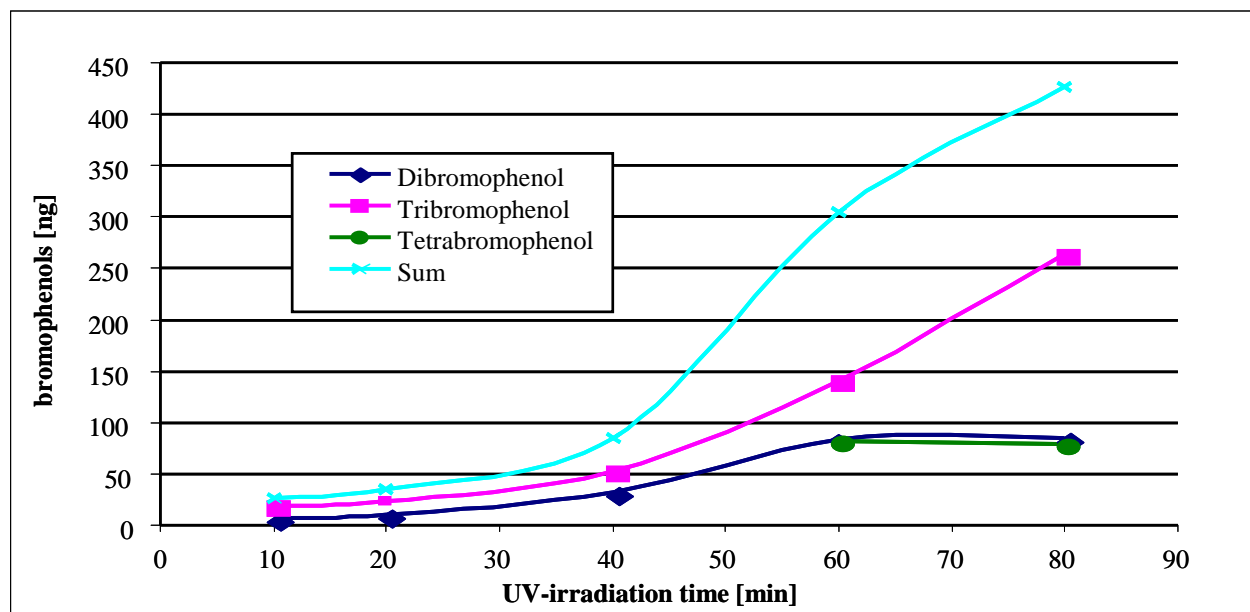
**Irradiation experiments and sample preparation.** Irradiation experiments were performed with a 400 μL solution of methanol containing 0.7-21 ng/μL BDE-154, -153, -183, -196, or a 1:1 mixture of BDE-197 and BDE-183 previously isolated by HPLC from DE-79<sup>11</sup>. The mixture of BDE-183/-197 (~17 ng/μL each) was evaporated to dryness and taken up in methanol-*d*<sub>4</sub>. Irradiations were performed in 0.5 cm path length quartz glass cuvettes placed in quartz glass beakers and cooled to 20 °C by a water bath. A SOL 500 sunlight simulator (400 W, Hönle, Gräfelfing, Germany) fitted with a UV-filter WG 295, λ > 280 nm (Schott, Mainz, Germany)

was used as the light source. Irradiations were performed for 0, 10, 20, 40, 60 and 80 min respectively. After the experiments, 20  $\mu\text{L}$  of internal standard solution (1.08  $\text{ng}/\mu\text{L}$  6-MeO-BDE-47) were added to 100  $\mu\text{L}$  aliquots. Additionally a 10  $\text{ng}/\mu\text{L}$  toluenic solution of DE-79 was irradiated for 20 min and a 400  $\text{ng}/\mu\text{L}$  solution of BDE-47 was irradiated for 20 min. For experiments with natural sunlight, a stock solution of 500  $\text{ng}/\mu\text{L}$  of technical OctaBDE (DE-79) in methanol were performed in 1 cm path length quartz glass cuvettes equipped with PTFE stoppers on a balcony in Stuttgart, Germany.

## Results and discussion

Screening for the BP-selected ions in GC-ECNI-MS verified the presence of BPs after a short time. In the case of BDE-183 the BP formation exponentially increased after 40 min of irradiation (**Figure 1**). TetraBPs were only detected in the exponential phase after 60 min of irradiation but the amounts did not increase after 80 min of irradiation. This could be explained by the fact that the precursor BDE-183 had almost disappeared at this point. Likewise, the formation of diBPs stopped after 60 min. All together, we observed one tetraBP, four triBPs and four-five diBPs (two peaks were observed for the coeluting 2,4-, 2,5- and/or 2,3-diBP) was observed after 80 min (**Figure 2**). The heptabromo congener BDE-183 has Br-substituents in 2,3,4,6- and 2,4,5-positions on the two ring moieties so that this molecule can produce seven di- to tetraBPs (**Table 1**). This corresponds with the maximum number of BPs detected in the sample (**Figure 2**). However the intensities of the BP congeners varied significantly. For instance, only traces of 2,6-diBP were detected (**Figure 2a**) which indicated that potential precursor PBDEs (2,6-substituted ring moieties) were not primary formed during the photolysis.

Irradiation of other PBDE congeners was also accompanied with the formation of BPs. For instance, irradiation of BDE-154 (2,4,5- and 2,4,6-substituted) generated the corresponding 2,4,5- and 2,4,6-triBPs while BDE-153 (2,4,5-substituted on each ring moiety) formed only the expected 2,4,5-triBP. Irradiation of BDE-196 generated the same pattern as observed for BDE-183 plus 3,5-diBP (**Table 1**).



**Figure 1.** Bromophenol formation during the UV-irradiation of 10500 ng BDE-183 in methanol

In contrast to the BPs observed after UV transformation of PBDEs, the pattern observed of naturally occurring BPs usually only features 2,4-diBP, 2,6-diBP and 2,4,6-triBP. Therefore, occurrence of other BPs e.g. the second and third most abundant 2,4,5-triBP and 2,3,6-triBP (**Figure 2b**) in environmental samples could potentially give indications, that these BPs would be metabolites of BFRs, such as PBDEs or pentaBP.

**Table 1:** Bromophenols which could be formed during the UV transformation of major PBDE congeners in octaBDE (DE-79)

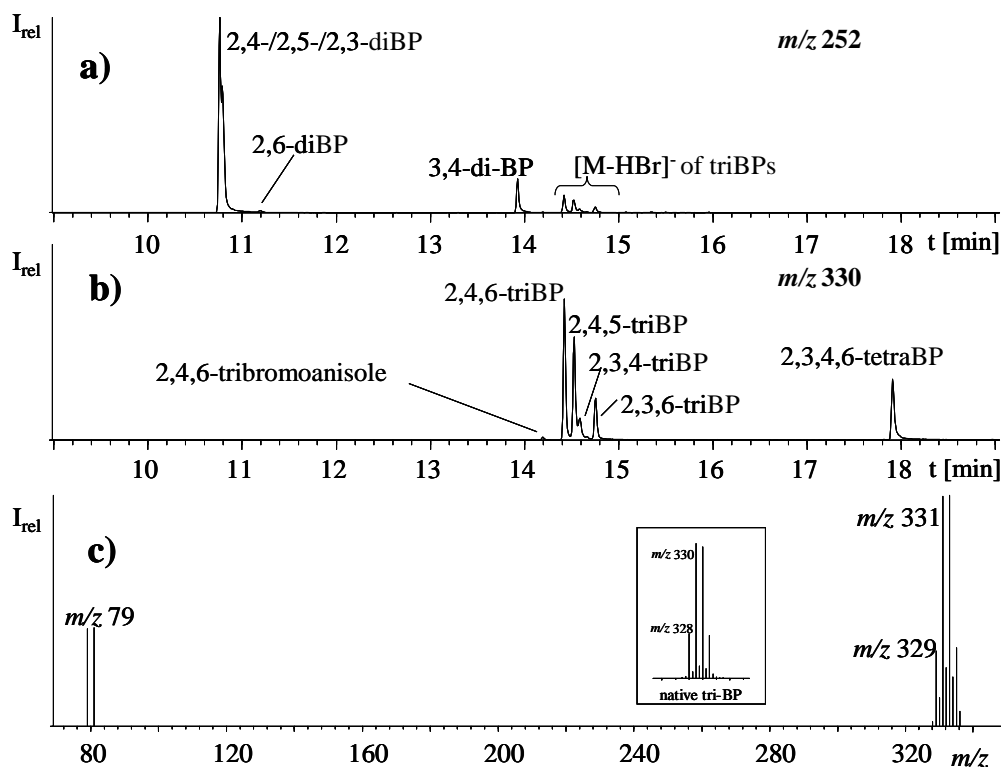
PBDE	BDE-153		BDE-154		BDE-183		BDE-196		BDE-197	
substitution	2,4,5	2,4,5	2,4,5	2,4,6	2,3,4,6	2,4,5	2,3,4,5	2,3,4,6	2,3,4,6	2,3,4,6
bromophenol										
2,3					x		x	x	x	x
2,4	x	x	x	x	x	x	x	x	x	x
2,5	x	x	x			x	x			
2,6				x	x			x	x	x
3,4	x	x	x		x	x	x	x	x	x
3,5							x			
2,3,4					x		x	x	x	x
2,3,5							x			
2,3,6					x			x	x	x
2,4,5	x	x	x			x	x			
2,4,6				x	x			x	x	x
3,4,5							x			
2,3,4,5							x			
2,3,4,6					x			x	x	x
2,3,5,6										

The UV-irradiation of 10.5  $\mu\text{g}$  BDE-183 for 80 min generated 425 ng BPs, i.e. ~4% of the initial amount. At this point only ~0.2% of BDE-183 was present in the solution and >90% was hydrodebrominated into hexa- to monoBDEs. Furthermore, traces of tribromoanisole were detected which was most likely formed as an artifact by the reaction of 2,4,6-triBP with the solvent methanol. GC/ECNI-MS full scan analyses gave also indications for the formation of bromobenzenes. The amount of BPs detected in this study was lower than the 17% 2,4,6-triBP formed from 2,3-dibromopropyl-2,4,6-tribromophenylether (DPTE)<sup>8</sup>. Obviously, the di-aromatic ethers (e.g. PBDEs) were more stable than aromatic-aliphatic ethers such as DPTE.

The irradiation of a mixture of BDE-183 and BDE-197 in methanol- $d_4$  and consecutive analysis of the isotope pattern with GC/ECNI-MS-SIM showed that the monoisotopic peak of the triBPs was shifted by one mass unit which originates from the deuterium addition to the oxygen. Likewise the abundance of the  $[M+1]^-$  signal at  $m/z$  330 was elevated compared to the native isotope pattern (**Figure 2c**). This increase in abundance emerges from the incorporation of one (additional) deuterium in the ring by reductive debromination. The higher abundance of  $m/z$  329 compared to  $m/z$  330 indicated that the 2,4,5-triBP was predominantly formed by BDE-183. In contrast the isotope patterns of the molecular ions of the diBPs were shifted by one more mass. The attachment of deuterium to the phenol proved that the hydrogens ( $^2\text{H}$ ) introduced in the both the hydrodebromination and ring cleavage reaction originated from the solvent. However, this experimental setup was not suited to elucidate if the hydrodebromination occurred exclusively with the PBDEs or also with the BPs.

Additional irradiation experiments showed that the formation of BPs was not only restricted to hexa- to octaBDEs. For instance, the irradiation of BDE-47 was accompanied with the formation of 2,4-diBP already after 20 min. Furthermore, the irradiation of DE-79 in benzene and toluene showed that the BPs formation was not depending on the presence of methanol. Also the conditions in the sunlight simulator proved to be realistic as exposure of a methanolic solution of DE-79 to natural sunlight resulted in BP formation.

As bromophenols were detected quite frequently in biotic degradation processes of PBDEs, we investigated samples of DE-71 and DE-79 which were hydrodebrominated with superreduced vitamin B<sub>12</sub><sup>12,13</sup>. However no BP formation was found to be mediated by this system.



**Figure 2.** GC/ECNI-MS-SIM ion chromatograms of (a)  $m/z$  252 for dibromophenols and (b)  $m/z$  330 for tri- and tetrabromophenols and (c) the GC/ECNI-MS-SIM mass spectrum (SIM-02) of 2,4,5-tribromophenol originating from BDE-183/197 in methanol- $d_4$

It should be noted that the analysis of the BPs required thoroughly deactivated inlet liners because any dirt in the liner immediately led to the disappearance of the BPs from the GC/ECNI-MS chromatograms. Additional problems arose from the GC stationary phase because several tested DB-5 like columns produced strong peak tailing. These instrumental challenges could be the reason that BPs have only been detected in one study of the photolysis of PBDEs (only after derivatization) <sup>9</sup>.

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