# Synthesis and partial characterization of a polybrominated dibenzofuran (PBDF) mixture

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## Introduction

Polybrominated dibenzofurans (PBDFs) are a group of toxic substances which are formed unintentionally and not for any commercial use. Nevertheless PBDFs have been measured in sediment samples[1] as well as aquatic organisms like mussels[2] and even in human adipose tissue[3]. PBDFs are present as impurities of large-scale brominated flame retardants (e.g. PBDEs, PBBs and TBBP-A)[4–6] and can be formed under thermal[6,7] or photolytical stress[8,9]. Therefore, PBDFs can enter the environment after incineration or accidental fires of plastic products like TV casings which have been treated with brominated flame retardants[7]. One HeptaBDF and one HexaBDF has also been detected after heating of salmon filet spiked with BDE-209[10,11]. There are 135 possible PBDF congeners, 10 of them with 2,3,7,8-substitution pattern which is directly linked to the toxic potential of this class of chemicals[12]. While the chlorinated dibenzofurans are well investigated and a lot of commercial standards are available, there is still a lack of information and availability on the brominated representatives[13].

The aim of our study was to synthesize a mixture of PBDFs and to get more information on their molecular structure. This mixture was characterized by GC/MS and aliquots were fractionated by counter-current chromatography (CCC). An isolated tetrabrominated congeners was characterized by NMR and aliquots were brominated and debrominated by UV irradiation to create further congeners with specific substitution patterns.

## Materials and Methods

*Chemicals and standards.* Dibenzofuran (p.a., ≥ 98%), elemental bromine (A.C.S. grade, ≥ 99.5%), 2,2,4-trimethylpentane (for pesticide residue analysis), iron powder (≥ 99.9%) and aluminum bromide (≥ 98%) were from Sigma-Aldrich (Steinheim, Germany). Dichloromethane (gradient grade for HPLC, ≥ 99.9%) and *n*-hexane (for pesticide residue analysis, ≥ 95.0%) were from Th. Geyer (Renningen, Germany) whereas toluene (residue grade), silica gel 60 (for column chromatography) and sodium thiosulfate (A.C.S. grade, ≥ 99.5%) were from Fluka (Steinheim, Germany). Demineralized water was produced in-house by means of an ELGA purelab classic ultrapure water system (Celle, Germany). Perdeuterated α-HCH (α-PDHCH) was used as internal standard[14]. A mix of 2,3,7,8-TetraBDF, 2,3,4,7,8- and 1,2,3,7,8-PentaBDF, 1,2,3,4,7,8-HexaBDF, 1,2,3,4,6,7,8,9-HeptaBDF and 1,2,3,4,6,7,8-OctaBDF (Camebridge Isotope Laboratories, Tewksbury, MA, USA) was kindly provided by CVUA Freiburg (Germany).

*PBDF synthesis.* Dibenzofuran (5 g, 29.8 mmol), aluminum bromide (0.5 g, 1.87 mmol), iron powder (0.5 g, 8.98 mmol) and 2 mL bromine (39.0 mmol) were placed in a 50 mL flask and heated for 60 min to 130 °C. After that, another 2 mL of bromine was added and the solution was heated for 120 min to 130 °C. The bulk of excess bromine was removed by distillation with a water cooled Liebig condenser at 80 °C. The remaining reaction mixture was dissolved in dichloromethane and treated with saturated sodium thiosulfate solution to remove remaining bromine. The organic phase was concentrated to 5 mL and further purified by silica gel column chromatography in a glass column (2.3 cm i.d.). The glass column was filled with silica gel deactivated with 30% water (21 g silica gel, deactivated with 9 g demin. water) covered with a 1 cm layer of water-free $Na_2SO_4$. PBDFs were eluted with 420 mL *n*-hexane followed by 100 mL *n*-hexane/toluene (95:5, v/v) and 100 mL toluene into one flask. After removal of the solvent, the final weight of the PBDF mixture was 5.6 g.

*Counter-current chromatography CCC.* Analyses were performed with an AECS Quickprep MK8 instrument (AECS, Downend, United Kingdom) equipped with a total coil volume of 471 mL and a 10 mL sample loop. Rotation speed was set at 865 rpm. The solvent system (*n*-hexane, acetonitrile and toluene, 45:45:10, v/v/v) was used in tail-to-head mode. The solvent was pumped by a ternary Beta 50 pump with a flow rate of 4 mL/min and UV signal was recorded with a Flash 10 diode array detector (both from Ecom, Praha, Czech Republic) at 231 nm. The synthesis mixture (350 mg), dissolved in the solvent system, was injected into the CCC system and the sample was fractionation by means of a Gilson 203B fraction collector (Middleton, WI, USA). After 51 min, 80 fractions of 7.5 mL were collected. The solvent of the fractions was evaporated by means of a gentle stream of nitrogen and residual was dissolved in 1 mL toluene. The internal standard α-PDHCH (11.7 ng in 10 µL *iso*-octane) was added before GC/EI-MS and GC/ECNI-MS measurements.

*Bromination of 1,3,6,8-TetraBDF*. In a 10 mL reaction tube 120 ng of 1,3,6,8-TetraBDF in 50 µL toluene was combined with 1 mg AlBr$_3$ and 1 mg iron powder. After removal of the solvent, 3 drops of bromine were added and the tube was closed and placed in an oil bath (130 °C) for 0, 5, 10 and 20 min. Excess bromine was removed by adding 1 mL saturated thiosulfate solution. Synthesis products were extracted with 1 mL toluene. The organic phase was removed and washed with bidest. water (3 times with 2 mL). The toluene phase was evaporated and re-dissolved in 1 mL toluene. A solution of the internal standard α-PDHCH (11.7 ng in 10 µL *iso*-octane) was added before GC/MS measurement.

*UV irradiation experiments*. Irradiation experiments in duplicate were carried out with 3.6 µg of 1,3,6,8-TetraBDF dissolved in 750 µL toluene filled in quartz cuvettes of 0.5 cm path length. The quartz cuvettes were placed in quartz glass beakers which in turn were placed in a water bath (25 °C) in front of a water-cooled mercury medium pressure TQ 150 lamp (Heraeus Noblelight, Hanau, Germany) without UV-filters. After 0, 10, 30, 60, 90, 120, 180, 300 and 600 s of irradiation, aliquots of 50 µL were taken. These aliquots were combined with a solution of the internal standard (11.7 ng in 10 µL *iso*-octane), made up to 1 mL and analyzed by GC/MS.

*Gas chromatography coupled to mass spectrometry (GC/MS)*. Measurements were performed with an Agilent 7890/5975c GC/MS system (Waldbronn, Germany) equipped with a Gerstel CIS-4 PTV injector (Mülheim, Germany) and an Agilent HP-5 MS UI column (30 m x 0.25 mm i.d. x 0.25 µm d$_f$). The PTV temperature program started at 80 °C (0.01 min), then ramped with 700 °C/min to 300 °C (2 min) and finally cooled with 10 °C/min to 260 °C until the end of the run. The GC oven programming started with 50 °C for 1 min and was raised with 10 °C/min to 300 °C (14 min). Helium (purity 5.0, Westfalen, Münster, Germany) was used as carrier gas with a constant flow rate of 1.2 mL/min. Measurements in electron-capture negative ion (ECNI) mode were performed with methane (purity 5.5, Air Liquide, Bopfingen, Germany) as reagent gas at 2 mL/min which resulted in a source pressure of 1.6 x 10$^{-4}$ Torr. 1 µL was injected and measurements were performed by electron ionization (EI-) and ECNI-MS in the full scan mode (*m/z* 50 – 800). Two-dimensional plots of CCC fractions over GC/MS runs were created with sigma-plot according to Kapp and Vetter[15].

## Results and Discussion

*Synthesis of polybrominated dibenzofurans*

The bromination of dibenzofuran (see experimental part) resulted in a mixture of ~40 out of 135 possible congeners with degrees of bromination ranging from one to seven (**Fig. 1a**). Di-, Tri- and TetraBDFs were the most relevant isomer groups with regard to peak abundances in GC/EI-MS mode and variety. The perbrominated OctaBDF could not be detected not even when a short 15 m GC column was used (data not shown). On the longer 30 m column, no overlapping of PBDFs with different bromination degree was noticed. However not all PBDF isomers were completely separated (**Fig. 1a**).
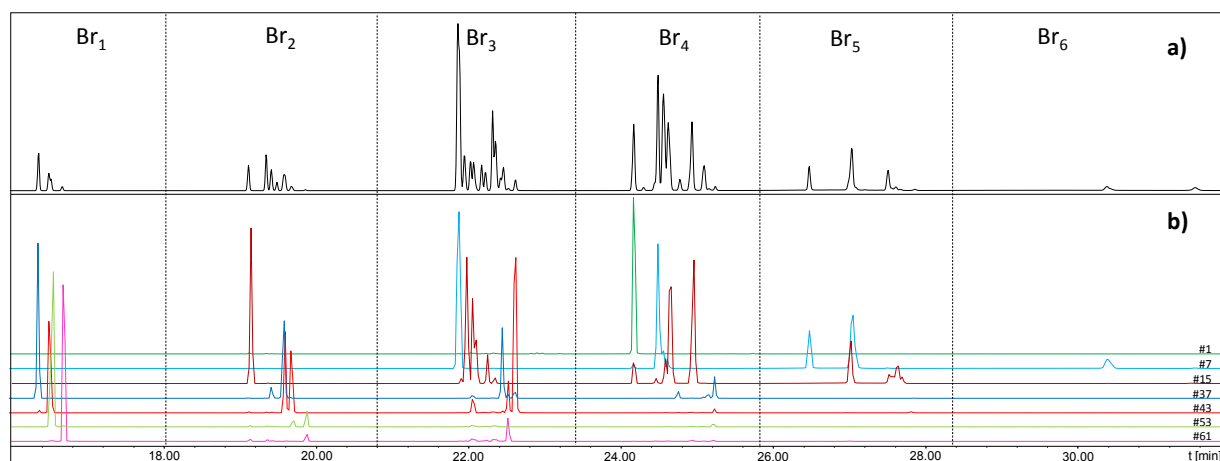


**Fig. 1:** GC/EI-MS full scan chromatograms of (a) the unfractionated PBDF synthesis mixture with approx. 40 detected PBDFs (one HeptaBDF congener is not shown) and (b) of CCC fractions #1, #7, #15, #37, #43, #53 and #61 resulting in less complex mixtures of PBDFs.

*Counter-current chromatography of the synthesis product*

Initial shake-flask experiments[15] with the solvent system *n*-hexane and acetonitrile[16] showed partition coefficients (K$_{U/L}$ values) suitable for CCC analysis but the solubility of PBDFs in this system was very low. For

this reason 10 vol% of toluene were added as modifier, which not only increased the sample capacity but also improved the $K_{U/L}$ values. This solvent system allowed to inject 350 mg PBDF mixture into the CCC system.

PBDFs started to elute after ~half the coil volume (235 mL) and the last PBDF eluted after 1.5 coil volumes (~707 mL). The first CCC fraction (#1, **Fig. 1b**) only contained one PBDF congener which was identified as 1,3,6,8-TetraBDF by ^{1}H-NMR. All subsequent fractions generally contained several PBDF congeners and isomers were usually distributed over several fractions. For example, the four MonoBDFs were detected in four CCC fractions (**Fig. 1b**). Typically, PBDFs with up to four consecutive degrees of bromination were detected in the individual fractions.

*2D contour plot of the CCC fractions over GC/MS analysis of the PBDF synthesis product*

Creation of a two-dimensional contour plot according to Kapp and Vetter[15] allowed to visualize >50 PBDF congeners (**Fig. 2**). However, isolation of further neat PBDFs could not be achieved. For example, early fractions (#5-30) were containing several overlapping di- and TriBDFs. The distribution of isomers over several CCC fractions allowed us to identify pairs of PBDFs which co-eluted in the GC/MS chromatograms (**Fig. 2**). For example, the isolated 1,3,6,8-TetraBDF (peak 4a, CCC fraction #1-7) had the same GC/MS retention time as a second TetraBDF isomer which eluted into CCC fractions #9-20. Noteworthy, 2,3,7,8-TetraBDF (peak **4b**, **Fig. 2**), which could be identified by means of a reference standard, was also co-eluting with another TetraBDF (peak **4c**, **Fig. 2**). Since 2,3,7,8-TetraBDF is most likely the most toxic PBDF congener[17], this GC problem could lead to an overestimation of the ΣTEQ if both isomers are present in samples.
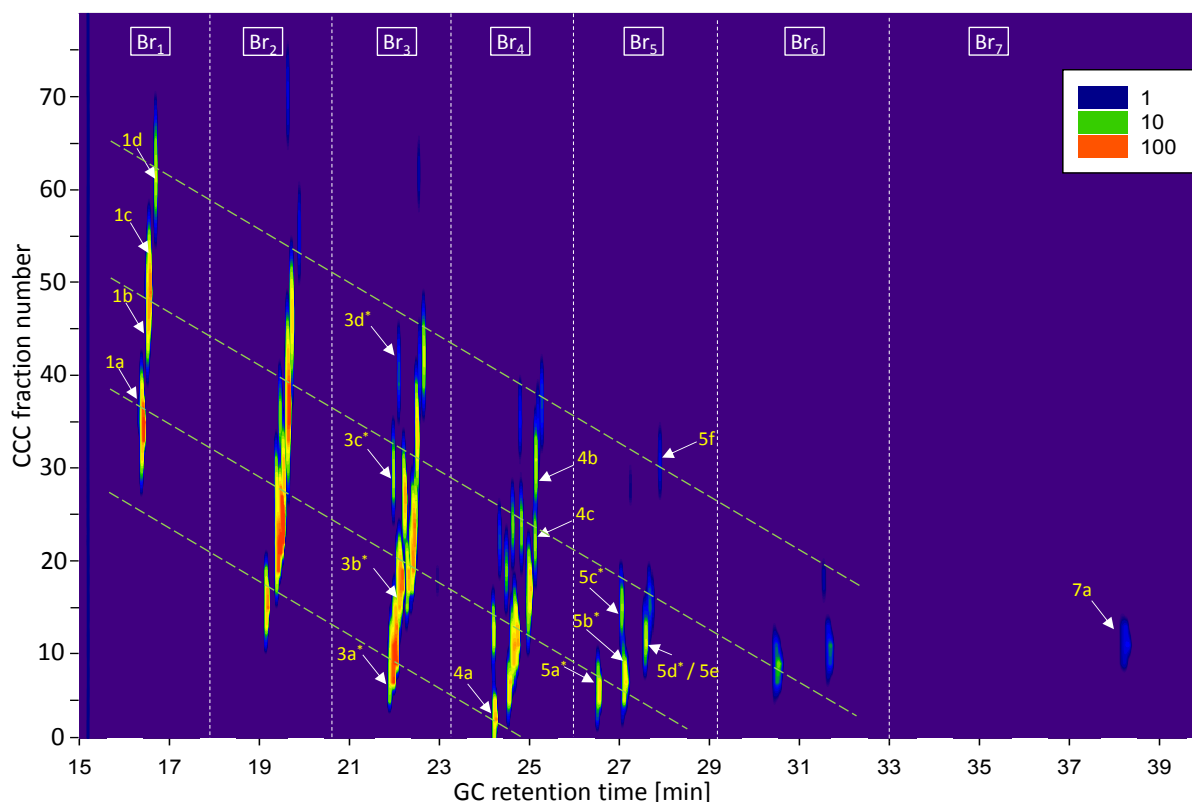


**Fig. 2:** Two-dimensional contour plot of CCC fraction number plotted against GC retention time of the synthesized PBDF mixture. Color codes were assigned relative to the signal abundance of the internal standard with $t_R$ = 15.2 min in the individual GC/EI-MS chromatograms. Identified or postulated (*) structures are marked with numbers for bromination degree + letters: **1a** (1-MonoBDF), **1b** (2-MonoBDF), **1c** (3-MonoBDF), **1d** (4-MonoBDF), **3a** (1,6,8-TriBDF), **3b** (2,4,7-TriBDF), **3c** (1,4,6-TriBDF), **3d** (1,4,8-TriBDF), **4a** (1,3,6,8-TetraBDF), **4b** (2,3,7,8-TetraBDF), **5a** (1,3,4,6,8-PentaBDF), **5b** (1,2,3,6,8-PentaBDF), **5c** (1,3,6,7,8-PentaBDF), **5d** (1,2,4,7,9-PentaBDF), **5e** (2,3,4,7,8-PentaBDF), **5f** (1,2,3,7,8-PentaBDF) and **7a** (1,2,3,4,6,7,8-HeptaBDF).Peak **4c** is a TetraBDF with unknown structure which is GC coeluting with **4b**.

*Bromination and debromination reactions with 1,3,6,8-TetraBDF*

*Bromination*. The controlled reaction led to the four possible PentaBDF isomers which can be formed from 1,3,6,8-TetraBDF by the addition of one Br (**Fig. 3**). The structures of the PentaBDFs were deduced from GC data of the corresponding chlorinated homologues[18]. Based on the assessment, PentaBDFs eluted in the order 1,3,4,6,8-PentaBDF (20% relative peak intensity) < 1,3,6,7,8-PentaBDF (18%) < 1,2,3,6,8-PentaBDF (major

isomer = 100%) < 1,2,4,7,9-PentaBDF (6%). Accordingly, bromination of 1,3,6,8-TetraBDF in 2-, 4- and 7-positions was superior to 9-position which is less likely due to steric crowding in case of Br in both 1- and 9-positions[19].

*UV irradiation*. Debromination of 1,3,6,8-TetraBDF led to four TriBDFs (i.e. all that can be formed by hydrodebromination) whose abundances increased with increasing irradiation time. Derived from GC data of PCDFs[20], TriBDFs eluted in the order 1,6,8-TriBDF (32% relative peak intensity) < 1,4,6-TriBDF (major isomer = 100%) < 1,4,8-TriBDF (10%) < 2,4,7-TriBDF (0.03%).

## Conclusions

Bromination of dibenzofuran led to a complex mixture of PBDFs with a great range of bromination degree. More than 50 out of 135 possible PBDFs were detectable. Isolation of 1,3,6,8-TetraBDF followed by UV irradiation and bromination reactions allowed to tentatively identify eight molecular structures (**Fig. 2**). CCC fractionation also allowed us to identify potential coelutions of 2,3,7,8-substituted PBDFs with less toxic ones.
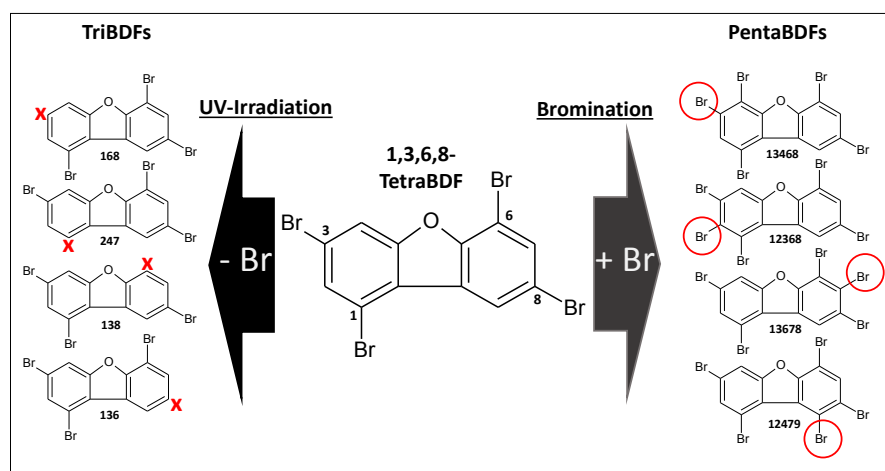


**Fig. 3:** Possible Tri- and PentaBDF structures resulting after irradiation or bromination of 1,3,6,8-TetraBDF. Numbers under the structures refer to the position of bromine atoms.

## References

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