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Analytical strategies for successful enantioselective separation of atropisomeric polybrominated biphenyls 132 and 149 in environmental samples

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Abstract

Some of the polybrominated biphenyls (PBBs) found in the environment are axially chiral, due to hindered rotation about the interannular phenyl–phenyl bond. This applies for PBB congeners having two or more bromine substituents in *ortho*-position to this bond. In this study analytical methods were developed that allow determining the enantiomer fraction (EF) of axially chiral (atropisomeric) PBBs in environmental samples. A white-tailed sea eagle egg was used as test sample. The egg extract was purified and further fractionated by normal phase (NP) high performance liquid chromatography (HPLC), yielding enriched fractions of axially chiral PBB 132 and PBB 149. Gas chromatographic (GC) enantioseparation of the atropisomers of PBB 149 was achieved on one of nine tested modified cyclodextrin phases. Due to coelution with an unknown brominated compound, conventional GC/ECNI-MS, which is based on the detection of the bromide ion, did not allow for the establishment of the EF. However, by means of GC/EI-MS-MS it was possible to verify an EF of 0.42–0.43, i.e. a significant enantiomeric enrichment of the second eluting atropisomer of PBB 149 in the white-tailed sea eagle egg. This is the first proof of non-racemic proportions of a chiral PBB in environmental samples. Despite the testing of nine different chiral stationary phases, GC enantioseparation of PBB 132 or other atropisomeric PBB congeners failed. For this reason, an enantioselective reversed-phase HPLC method was developed. This method proved to be a powerful tool for the separation of PBB atropisomers. It was found that even a standard of the di-*ortho* substituted PBB 153 could be partially separated into atropisomers at 0 °C but already enantiomerized at 5 °C. For establishing the EF of PBB 132 in the bird egg sample a combination of enantioselective HPLC followed by non-chiral gas chromatography was employed. Using enantioselective HPLC, the atropisomers of PBB 132 were quantitatively targeted into two separate fractions at room temperature (20 °C). After addition of internal standards for volume adjustment the relative amounts of the atropisomers in the isolated fractions were quantified by using non-chiral GC/EI-MS analysis. A deviation from the racemic mixture of the atropisomers of PBB 132 in the egg extract could not be statistically proven.

Keywords: Enantiomer separation; Atropisomerism; Firemaster BP-6®; Polybrominated biphenyls; Modified cyclodextrins; White-tailed sea eagle egg

1. Introduction

Polybrominated biphenyls (PBBs) have been extensively applied as flame-retardants in textiles, electronic equipment and plastics [1]. Technical PBB products have been marketed under trade names such as Firemaster BP-6®, Firemaster

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FF-1®, Bromkal 80® and Flammex-B®. Due to their structural analogy to polychlorinated biphenyls (PCBs), PBBs came into the spotlight of environmental research as early as in the 1970s [2,3]. Furthermore, in 1973, an accidental contamination of human food with PBBs in Michigan revealed the toxicological threat of this group of chemicals [4,5]. Despite a continuous reduction of the worldwide annual production since the late 1970s and slightly declining levels in the environment in the last decade, the ubiquitous presence of PBBs has been documented in a wide range of samples [6–9].

PBBs exist in a theoretical variety of 209 congeners [1]. Several PBB congeners, containing two to four bromine *ortho*-substituents, cannot rotate about the interannular phenyl–phenyl bond due to steric hindrance. Such PBB congeners, which additionally possess non-symmetric substitution patterns on both aromatic rings, form pairs of axially chiral compounds (atropisomers) [10,11]. Analogously, 19 axially chiral PCBs proved to form stable atropisomers under environmental conditions [12,13]. However, due to the more bulky bromine substituent it was assumed that more than 19 PBB congeners would form stable atropisomers at physiological temperatures [11]. Six axially chiral PBBs, including PBB 132 and PBB 149 (Fig. 1) were recently enantioseparated by means of high performance liquid chromatography (HPLC). In addition, the enantiomers of PBB 149 were partially resolved by enantioselective gas chromatography (GC) [11].

In this study, methods were developed providing analytical data on PBB enantiomers in environmental samples. The methods were applied to an egg of a Norwegian white-tailed sea eagle, representing a biological matrix with relatively high PBB contamination [6]. Results from enantioselective investigations of chiral compounds may add valuable information on the bioavailability and accumulation of anthropogenic contaminants in the food web [10,14]. This is due to the fact that chiral contaminants originally applied as racemates (enantiomer fraction (EF) = 0.5 or enantiomer ratio = 1.0) may undergo preferable depletion/accumulation of one enantiomer in biological systems [10,14]. Thus, a deviation of the EF from 0.5 in environmental samples is a direct indicator for bioactivity of the given chiral compound. Today, information about enantiomeric distribution in environmental samples exists for a large number of chiral chemicals including atropisomeric PCBs [15–18]. However, no studies investigating the fate of atropisomeric PBBs in the environment have been published so far.

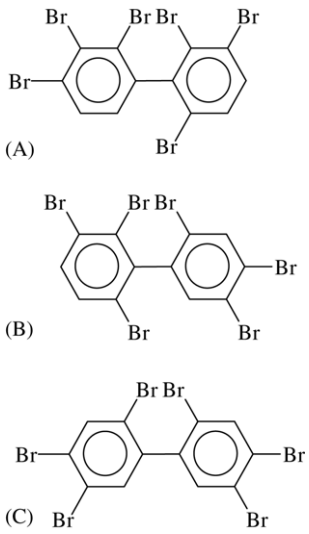

Fig. 1. Chemical structures of 2,2′,3,3′,4,6′-hexabromobiphenyl (PBB 132; A), 2,2′,3,4′,5′,6-hexabromobiphenyl (PBB 149; B), and 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB 153; C).

2. Experimental

2.1. Chemicals and samples

Technical hexabromobiphenyl (Firemaster BP-6®, 10 mg, Michigan Chemicals) was purchased from Promochem (LGC Promochem, Borås, Sweden). 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB 153, 5 mg) was supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Other single PBB congeners and enantiomers were previously prepared [11]. PCB 138, PCB 153, PCB 180, PBDE 77, *p*,*p*′-DDE, HCB, β-HCH, heptachlor, *trans*-nonachlor, and *cis*-nonachlor were used for method development and octachloronaphthalene (OCN) as well as $^{13}C_{12}$-PBDE 77 were used as internal standards (see later). These compounds were obtained from Promochem or, alternatively, from Cambridge Isotope Laboratories (Woburn, MA, USA). Acetonitrile (for HPLC), *n*-hexane (for GC), isooctane (for GC), ethyl acetate (for organic trace analysis), and silica gel 60 (particle size 0.063–0.200 mm, 70–230 mesh ASTM) were obtained from Merck (Darmstadt, Germany). Water was purified with a Milli-Q system (Millipore SAS, Molsheim, France). Helium carrier gas (quality 6.0, Hydrogas, Porsgrunn, Norway or, alternatively, quality 5.0, Linde, Höllriegelskreuth, Germany), methane reactant gas (quality 5.5, Hydrogas or 5.0, Linde), nitrogen make-up gas (5.0, Linde) and argon collision gas (4.5, Linde) were used for gas chromatographic and mass spectrometric analyses. A non-hatched white-tailed sea eagle egg was analyzed. The egg was collected in Vikna, Nord-Trøndelag (Norway) after the hatching period in 1998 by the Norwegian Institute for Nature Research, Trondheim (Norway). The hen's egg used for method development (see below) was purchased in a local Norwegian supermarket.

2.2. Sample preparation

Two separate aliquots of the same egg sample were extracted and cleaned-up. Homogenization, drying, and cold column extraction of about 20 g (one aliquot) of the bird egg as well as clean-up of the extract by GPC and a florisil column

were performed according to Herzke et al. [6]. For separation of brominated compounds from PCBs, the purified sample extract in isooctane was concentrated in a Turbovap evaporator (Zymark, Hopkinton, MA, USA) to approximately 0.5 mL and fractionated on 8 g activated silica adapting a method published elsewhere [19]. The fractionation was optimized with a test mixture containing *p,p′*-DDE, PCB 138, PCB 153, PCB 180, PBDE 77, and Firemaster BP-6® (technical mixture) and tested with the extract of a hen's egg spiked with the same test mixture. No effects of the egg matrix on the fractionation were observed. The bulk of the PCBs eluted with 48 mL *n*-hexane in the first fraction, which was discarded. Brominated compounds were then eluted with another 10 mL *n*-hexane plus 50 mL *n*-hexane/ethylacetate (9/1, v:v). This combined fraction also contained most organochlorine pesticides [19]. Isooctane (1 mL as keeper) was added and the fraction was concentrated in the Turbovap system and under nitrogen to a final volume of approximately 150 μL.

2.3. Fractionation by normal phase HPLC

Normal phase (NP) HPLC was used for the separation of PBBs from most of the PBDEs and organochlorine pesticides. The HPLC system consisted of a Waters 616 low-pressure quaternary gradient pump (Waters, Milford, MA, USA), a manual six-port injection valve equipped with a 200 μL loop (Rheodyne, Rohnert Park, CA, USA) and a Waters 996 photodiode array detector (scanning 190–250 nm). A silica precolumn (10 mm × 3 mm i.d., Varian, Palo Alto, CA, USA) and two silica HPLC columns (250 mm × 4.6 mm i.d., 5 μm particles, Varian and 200 mm × 4 mm i.d., 5 μm particles, 100 Å pore size, Nucleosil®, Macherey–Nagel, Düren, Germany) were coupled in series. The separation was performed at room temperature employing an isocratic flow of 1 mL/min *n*-hexane. A test mixture containing HCB, β-HCH, heptachlor, *trans*- and *cis*-nonachlor, *p,p′*-DDE, PBDE 77 and Firemaster BP-6® was used for method development and again the spiked hen's egg extract was analyzed for evaluation of matrix effects. A slight shift towards longer retention times was observed in presence of the egg matrix, despite the exhaustive clean-up it had undergone. The 150 μL extract from silica fractionation were injected and the following fractions were collected manually: 3–7 min, 7–8 min, 8–9.5 min, 9.5–10.5 min, 10.5–11.5 min, and 11.5–30 min (see also Section 3.1). These fractions were first concentrated to approximately 100 μL for GC-MS evaluation. The fraction containing PBB 149 was then concentrated to 50 μL for enantioselective GC analysis and the fraction with PBB 132 was gently evaporated to dryness and redissolved in 150 μL acetonitrile/water (60/40, v/v) for enantioselective HPLC separation.

2.4. Enantioselective HPLC

Enantioselective HPLC was performed on a system consisting of a Waters 515 HPLC pump, a manual Rheodyne six-port injection valve equipped with a 200 μL loop (sample volumes ≤150 μL injected) and a Waters 2487 UV detector set to 220 nm. Atropisomers of PBB 132 and PBB 153 were separated on a Nucleodex [β]-PM column (β-PMCD; heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin on silica, 200 mm × 4 mm i.d., Macherey–Nagel) employing an isocratic flow of 0.5 mL/min acetonitrile/water (60/40, v/v). For partial separation of PBB 153 atropisomers the solvent container and the column were cooled to 0 °C in an ice bath. In contrast, enantiomers of PBB 132 were separated at room temperature (20 °C) and five fractions (12–17 min, 17–22.5 min, 22.5–24 min, 24–35 min, and 35–40 min) were collected manually (see also Section 3.2). The middle fraction (22.5–24 min) was concentrated under nitrogen to approximately 50 μL (mainly water remaining), taking advantage of the minimum azeotrope (boiling point 76.5 °C) of acetonitrile/water (85/15, v/v). A volume of 50 μL acetonitrile was added and the solution was again manually fractionated on the β-PMCD HPLC column using the same retention time windows as before. The corresponding fractions of the first and second fractionation were combined and 2 ng $^{13}C_{12}$-PBDE 77 (in 20 μL acetone) was spiked to each of these combined fractions. Then they were extracted five times with 2 mL *n*-hexane and the combined extracts were concentrated to approximately 50 μL. After addition of 4 ng OCN (in 20 μL isooctane) the fractions were concentrated to a final volume of 20 μL for GC-MS quantification (see Section 2.5).

2.5. Non-chiral GC-MS

Non-chiral GC-MS was performed using a Mega II 8065 gas chromatograph (Fisons, Milan, Italy) coupled to an MD800 low-resolution quadrupole mass spectrometer (Finnigan, San Jose, CA, USA) operated in the electron ionization mode (GC/EI-MS). Samples (2 μL, solvent *n*-hexane or isooctane) were injected on-column (AS800 auto-injection system, Fisons) onto a DB5MS capillary column (phenyl arylene polymer, 30 m length, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Separations were performed using helium carrier gas at a constant column head pressure of 80 kPa. The GC oven was programmed as follows: 70 °C (hold time 2 min), then at 15 °C/min to 180 °C, then at 5 °C/min to 280 °C (hold time 5 min), and finally at 5 °C/min to 300 °C (hold time 7 min). The GC-MS interface and EI ion source temperatures were hold at 250 and 200 °C, respectively. The mass spectrometer was operated in single ion monitoring (SIM) mode. For evaluation of NP-HPLC fractions the following mass to charge ratios were monitored: *m/z* 547.6 and 549.6 (pentabromobiphenyls), *m/z* 625.6 and 627.6 (hexabromobiphenyls), *m/z* 705.5 and 707.5 (heptabromobiphenyls), *m/z* 283.8 and 285.8 (HCB), *m/z* 216.9 and 218.9 (β-HCH), *m/z* 271.8 and 273.8 (heptachlor), *m/z* 406.8 and 408.8 (*trans*- and *cis*-nonachlor), *m/z* 246.0 and 248.0 (*p,p′*-DDE), *m/z* 483.7 and 485.7 (PBDE 77). For quantification of PBB 132 atropisomers in fractions from enantioselective

HPLC the following ions were monitored: *m/z* 625.6 and 627.6 (hexabromobiphenyls), *m/z* 403.8 and 405.8 (OCN), *m/z* 495.8 and 497.8 ($^{13}C_{12}$-PBDE 77).

2.6. Testing of enantioselective GC columns

Studies on the enantioseparation of PBB 149 and PBB 132 were performed by gas chromatography (HP 5890 series II, Agilent, Palo Alto, CA, USA) with a ^{63}Ni electron capture detector (GC/ECD). Nine columns with different chiral stationary phases (CSPs) were tested (see below). Standards (1 μL in *n*-hexane or isooctane) were injected splitless at an injector temperature of 250 °C. After initial tests with slow heating ramps the enantiomer separation was performed isothermal (after injection at 80 °C and heating at 15 °C/min to the target temperature) and optimized by stepwise lowering the isothermal elution temperature. The column head pressure was varied between 60 and 160 kPa.

The following GC columns were tested for separation of the atropisomers of PBB 132 and PBB 149 standards previously isolated from the technical product [11]: (1) 20 m β-TBDM column, 0.25 mm i.d., 0.15 μm film thickness (BGB Analytik, Adliswil, Switzerland); (2) 10 m β-BSCD column, 0.25 mm i.d., 0.23 μm film thickness (BGB Analytik); (3) 25 m Chirasil-Dex γ-PMCD column (chemically bonded to CP-Sil 5), 0.25 mm i.d., 0.25 μm film thickness (Varian Chrompack, The Netherlands); (4) 30 m polysiloxane diluted γ-PMCD column, 0.25 mm i.d., 0.25 μm film thickness (γ-dex 120, Supelco, Taufkirchen, Germany); (5) 25 m FS-LIPODEX column, 0.25 mm i.d., 0.25 μm film thickness (Macherey–Nagel, Düren, Germany); (6) 15 m β-PMCD column, 0.25 mm i.d., 0.25 μm film thickness (shortened β-dex 120, Supelco); (7) 15 m β-TBDM column (0.25 mm i.d., 0.25 μm film thickness, F0 fraction [20]; (8) 15 m β-TBDM column (0.25 mm i.d., 0.25 μm film thickness, F2 fraction [20]); (9) 15 m β-TBDM column (0.25 mm i.d., 0.25 μm film thickness, F5 fraction [20]). Columns 7–9 were lab-made (not commercially available) from fractions obtained from LC-fractionation of the β-TBDM phase, which was also used in column 1. It was previously shown that the β-TBDM phase was a mixture of at least six major products. For this reason, the raw product was LC-fractionated as described by Ruppe et al. [20]. Fractions were labeled in order with increasing polarity, and fractions F0, F2, and F5 gave best results for some individual organochlorine compounds.

2.7. Enantioselective GC/EI-MS-MS analysis of PBB 149

GC/EI-MS-MS experiments were performed with a CP-3800 gas chromatograph interfaced to a 1200 triple quadrupole MS/MS system (Varian, Darmstadt, Germany). Samples were introduced with a CP-8410 auto injector (Varian). A β-TBDM column (no. 1, see above) was installed in the GC oven. Helium was used as carrier gas at a constant flow of 1 mL/min. The GC oven temperature for PBB 149 started at 80 °C (hold time 1 min), was then raised at 20 °C/min to 190 °C (hold time 93.5 min), and finally at 5 °C/min to 210 °C/min (hold time 26 min). The total run time was 130 min. The injector and transfer line temperatures were both set to 220 °C. Samples of 1 μL (splitless time 4 min) were injected using an initial pressure pulse for 4 min at 15 psi. Mass spectra were recorded in the selected reaction monitoring (SRM) mode applying a source temperature of 200 °C, an electron energy of 70 eV, and a filament emission current of 150 μA. Fragmentation of the most abundant $[M]^+$-isotope signal at *m/z* 627.6 ($[C_{12}H_4{}^{79}Br_3{}^{81}Br_3]^+$) to the most abundant $[M-Br]^+$ isotope signal at *m/z* 546.7 ($[C_{12}H_4{}^{79}Br_3{}^{81}Br_2]^+$) was performed at a collision cell pressure of ∼1 mTorr (argon) and a collision voltage of 20 V. The detector voltage was set to 2000 V. More details are reported elsewhere [21].

3. Results and discussion

3.1. Liquid chromatographic enrichment of PBB 132 and PBB 149

After a general clean-up procedure for organohalogens in extracts from bird egg samples [6], two fractionation steps were employed in order to separate PBBs from most other organohalogen compounds, which could have impaired the enantioselective analyses. PCBs were separated from PBBs on deactivated silica (see Section 2.2). The collected fraction quantitatively contained all investigated PBBs. Only trace amounts of PCBs were left, however, PBDEs and most organochlorine pesticides have been shown to coelute with the PBBs [19]. Therefore, the concentrated sample extract was further fractionated using silica HPLC (Fig. 2). This technique allowed separating PBB 132 and PBB 149 quantitatively into two fractions (Fig. 2, fractions 8–9.5 min and 9.5–10.5 min). This was important, since these two congeners had to be treated separately in enantioselective separation. The total extracted amounts of PBB 132 and PBB 149 were 12 ng and 3 ng, respectively (based on the response factor of PBB 153). Furthermore, HCB, β-HCH, the nonachlor isomers, and PBDE 77 were separated from the PBBs. Of the standards tested, besides other PBB congeners only heptachlor and *p,p′*-DDE partly eluted into the fractions where PBB 132 and PBB 149 were found. These fractions were concentrated and used for enantioselective studies.

3.2. Enantioselective separations of PBB 132 and PBB 153 by HPLC

Despite the testing of nine different CSPs, the enantioselective GC separation of PBB congeners other than PBB 149 failed. Therefore, additional experiments were performed with enantioselective HPLC, which proved to be a powerful tool for the chromatographic enantioseparation of many PBBs [11]. Previous studies showed that lowering the

Compound	Distribution of organohalogen compounds in NP-HPLC fractions (mean value of two separations of the extract from a spiked hen's egg) 3-7 min	7 - 8 min	8 - 9.5 min	9.5 - 10.5 min	10.5 – 11.5 min	11.5 – 30 min
HCB	■	▒				
β-HCH						■
heptachlor			▒	■	▒	
trans-nonachlor						■
cis-nonachlor						■
p,p'-DDE			■	▒		
PBDE 77						■
PBB 101			■			
PBB 132			■			
PBB 138			▒	■		
PBB 149				■		
PBB 153			■	▒		
PBB 174			■			
PBB 180			■			

□ <limit of detection (LOD) ▒ LOD - 20 % ■ 80 - 100 %

Fig. 2. Separation of PBBs from most PBDEs and organochlorine pesticides by normal phase (NP) HPLC in the extract of a spiked hen's egg (for more details see text).

temperature of the β-PMCD HPLC column to 5 °C led to a very broad peak of the di-*ortho* substituted PBB 153, indicating a slow conversion from one enantiomeric form into the other [11]. In fact, when the HPLC column was cooled to 0 °C, PBB 153 enantiomers could partly be resolved (data not shown), even though there are no "buttressing" bromine substituents in adjacent *meta*-positions to the two *ortho*-bromines (see also [11,22]).

In contrast to PBB 153, baseline separation of the atropisomers of the tri-*ortho* substituted PBB 132 was easily achieved employing the β-PMCD column at room temperature (Fig. 3A). Unfortunately, PBB 153 coeluted with the first eluting atropisomer of PBB 132 in the enantioselective HPLC separation. Since PBB 132 and PBB 153 were present in the same fraction of the egg extract (see Fig. 2), a direct HPLC/UV quantification of the PBB 132 enantiomers was not possible. Therefore, an enantioselective HPLC fractionation of PBB 132 with consecutive non-chiral GC-MS quantification of the isolated PCB 132 atropisomers was attempted. Initial experiments to establish retention time windows were performed with a standard solution of PBB 132 previously isolated from the Firemaster BP-6® mixture [11]. To control the quantitative collection of the atropisomers, a fractionation method with five fractions was elaborated, of which the 2nd and 4th contained the PBB 132 enantiomers, respectively, while fractions 1, 3, and 5 should be free of any PBB 132 (Fig. 3A). According to this method (see Section 2.4) the egg extract was injected and fractionated. Since an aqueous mobile phase was used in HPLC, the fractions had then to be extracted with *n*-hexane for GC analysis. To control the efficiency of this step and to be able to correct for injection volume variability as well as sensitivity fluctuations of the MS instrument, internal standards were added to the fractions before extraction ($^{13}C_{12}$-PBDE 77) and after extraction (OCN), respectively (see Section 2.4). The HPLC fractions were five times extracted and the five extracts were pooled. A sixth extraction of HPLC fractions 2 and 4 was free of PBB 132 and $^{13}C_{12}$-PBDE 77 and thus discarded.

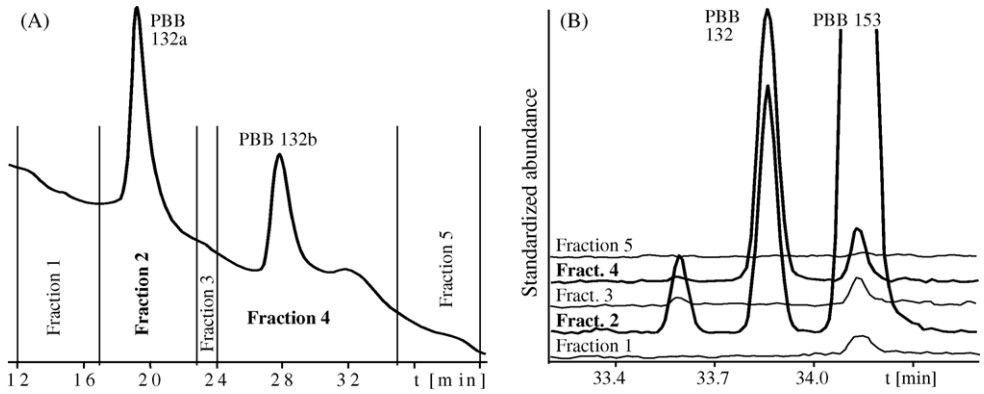

Fig. 3. (A) HPLC/UV chromatogram (220 nm, room temperature) of the enantioseparation of a PBB 132 reference standard isolated from the technical mixture. Five fractions collected for subsequent GC-MS analyses are indicated. (B) GC/EI-MS mass chromatograms (SIM mode, *m/z* 625.6) of the five fractions obtained from enantioselective HPLC separation of the white-tailed sea eagle egg extract.

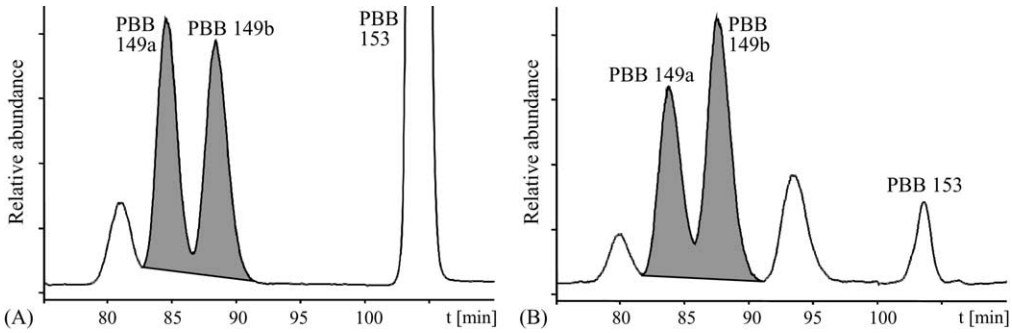

Fig. 4. Enantioselective GC/EI-MS-MS determination (for details see text) of PBB 149 in the technical mixture Firemaster BP-6® (A) and in the extract of the white-tailed sea eagle egg (B).

GC/EI-MS SIM-chromatograms of the pooled fraction extracts of the bird egg are shown in Fig. 3B. It can be seen that fractions 2 and 4 quantitatively contained the PBB 132 atropisomers, respectively, whereas fractions 1, 3, and 5 were free of PBB 132. Quantification of the noise in the chromatograms revealed that the detection limits of PBB 132 in fractions 1, 3, and 5 were <1% relative to the peak areas of fractions 2 and 4. These latter fractions were analyzed four times by GC-MS and the absolute signal areas of the PBB 132 enantiomers in fractions 2 and 4 were corrected relatively to the signal area of one of the internal standards. The calculated EF of PBB 132 (first eluting enantiomer on the HPLC column) was 0.48–0.50 ($n = 4$; correction with $^{13}C_{12}$-PBDE 77) or 0.47–0.50 ($n = 4$; correction with OCN). These results might indicate a minor relative enrichment of the second eluting enantiomer of PBB 132. However, due to the variability of the method (especially consecutive GC-MS analyses), the deviation from the racemic mixture was not significant.

3.3. Enantioselective separation of PBB 149 in the egg extract by GC/EI-MS-MS

Among the nine CSPs tested only β-TBDM enabled a sufficient resolution of PBB 149 atropisomers. The column used in the present study provided a slightly better enantiomer resolution than was previously obtained with a similar column [11]. This is most likely due to minor batch-to-batch variations, which are known to occur for this kind of CSP [23]. Unfortunately, when applying conventional GC/ECNI-MS in the SIM mode, monitoring of the bromide ions *m/z* 79 and *m/z* 81 was not selective enough for determination of the EF of PBB 149 in the sample. An interfering brominated compound was detected which eluted between the separated PBB 149 enantiomers. Since the interfering compound was not present in Firemaster BP-6®, it appeared not to be a PBB congener. Although PBDE 77 was well separated from the PBB fraction in silica HPLC (Fig. 2), it could not be excluded that other PBDE congeners were still present in the fraction containing PBB 149. Other candidates for the co-eluting organobromine compound might be halogenated natural products, which also were identified in comparable samples [6]. For this reason the major halogenated natural product BC-2 and technical pentabromodiphenyl ether (DE 71) were analyzed on the chiral β-TBDM column. The PBB 149 atropisomers eluted after the tetra- and pentaBDEs and BC-2 but before the hexaBDEs. None of the PBDE congeners with abundance higher than 0.5% in the DE 71 mixture was the interfering compound, and its structure could not be elucidated. However, using GC/EI-MS-MS solved the problem with the interference. With this more selective detection method, no interference was found for both, technical Firemaster BP-6® and the egg extract (Fig. 4). Repetitive injections of PBB 149 enantiomers in both standard and sample showed slight variations in the retention times (±8 s), which was considered acceptable in view of the long retention times. Furthermore, the α-values in both standard ($\alpha = 1.044$–1.048; $n = 10$) and sample ($\alpha = 1.045$–1.046; $n = 3$) agreed very well. The EF for PBB 149 in Firemaster BP-6® was 0.49–0.54 ($n = 10$; Fig. 4A). Enantioselective GC/EI-MS-MS analysis of the bird egg extract confirmed the relative enrichment of the second eluting atropisomer of PBB 149 (Fig. 4B). For establishing the EF of PBB 149 in the white-tailed sea eagle egg, two separate aliquots of the same egg sample were extracted, cleaned up and analyzed independently. GC/EI-MS-MS measurements resulted in an EF for the first eluting atropisomer of 0.42–0.43 ($n = 3$) for both extracts. The signal-to-noise ratio of the PBB 149 enantiomers was >20:1 so that the integration could be carried out with high accuracy. Therefore, these results indicate a significant enantioselective enrichment of PBB 149 in this predatory bird egg from Norway. It is also noteworthy, that there was a hexabromobiphenyl congener present in the egg extract, eluting just after the PBB 149 atropisomers (Fig. 4B), which was not present in the technical PBB mixture (Fig. 4A). Such congeners have also been observed in other Norwegian bird of prey eggs [6]. They may originate from applications/uptake from other technical products or they might be degradation products of higher brominated biphenyls.

4. Conclusions

Enantioseparation of PBB atropisomers in both standards and environmental samples remains a technical challenge.

Despite new efforts only the enantiomers of PBB 149 could be separated using gas chromatography. GC/EI-MS-MS proved to be a powerful tool providing sufficient selectivity and sensitivity for an interference-free, direct quantitative determination of the EF of PBB 149. This was demonstrated by analyzing a predatory bird egg, where a non-racemic composition of PBB 149 was found. This is the first report on enantioselective PBB data in environmental samples.

Direct determination of the EF of PBB 132 in an environmental sample could not be achieved, neither by GC-MS nor HPLC/UV techniques. However, an analytical method was developed allowing enantioselective studies of PBB 132 by a combination of HPLC fractionation of the atropisomers followed by GC-MS quantification of the volume-adjusted fractions. This novel approach was successfully applied to the predatory bird egg sample, but did not give a clear picture whether or not PBB 132 was present in a non-racemic mixture. Nevertheless, it is clear that the EF of PBB 149 in the bird egg differed much more from the racemate than the EF of PBB 132. Quantitative chiral HPLC fractionation of enantiomers followed by non-chiral GC-MS determination of the relative concentrations is a promising tool for establishing enantioselective data for compounds that cannot be enantioseparated by GC. The here presented results indicate that with this technique, enantioselective enrichment can be proven when one enantiomer is at least 10% more abundant than its mirror image.

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References

[1] J. de Boer, K. de Boer, J.P. Boon, in: K. Part, J. Paasivirta (Eds.), The Handbook of Environmental Chemistry, vol. 3, Springer-Verlag, Berlin, Heidelberg, 2000, p. 61.
[2] Å. Norström, K. Andersson, C. Rappe, Chemosphere 5 (1976) 255.
[3] G. Sundström, O. Hutzinger, S. Safe, V. Zitko, Sci. Total Environ. 6 (1976) 15.
[4] S.M. Getty, D.E. Rickert, A.L. Trapp, CRC Crit. Rev. Environ. Control (1977) 309.
[5] K. Kay, Environ. Res. 13 (1977) 74.
[6] D. Herzke, U. Berger, R. Kallenborn, T. Nygård, W. Vetter, Chemosphere (2005) in press.
[7] Proceedings of the Second International Workshop on Brominated Flame Retardants, The Swedish Environmental Protection Agency, May 14–16, 2001.
[8] Proceedings of the Third International Workshop on Brominated Flame Retardants, University of Toronto, Toronto, Ontario, Canada, June 6–9, 2004.
[9] M. Alaee, L. Luross, D.B. Sergeant, D.C.G. Muir, K. Solomon, D.M. Whittle, Organohal. Compd. 40 (1999) 347.
[10] R. Kallenborn, H. Hühnerfuss, Chiral Environmental Pollutants, Springer-Verlag, Berlin, Heidelberg, 2001.
[11] U. Berger, W. Vetter, A. Götsch, R. Kallenborn, J. Chromatogr. A 973 (2002) 123.
[12] K.L.E. Kaiser, Environ. Pollut. 7 (1974) 93.
[13] H.-J. Lehmler, L.W. Robertson, Atropisomers of PCBs, in: Robertson, Hansen (Eds.), PCBs – Recent Advances in the Environmental Toxicology and Health Effects, University of Kentucky Press, Kentucky, 2001, p. 61, (Chapter 8).
[14] W. Vetter, V. Schurig, J. Chromatogr. A 774 (1997) 143.
[15] W. Vetter, U. Klobes, K. Hummert, B. Luckas, J. High Res. Chromatogr. 20 (1997) 85.
[16] H. Hühnerfuss, B. Pfaffenberger, B. Gehrcke, L. Karbe, W.A. König, O. Landgraff, Mar. Pollut. Bull. 30 (1995) 332.
[17] A. Glausch, G.J. Nicholson, M. Fluck, V. Schurig, J. High Res. Chromatogr. 17 (1994) 347.
[18] A. Glausch, J. Hahn, V. Schurig, Chemosphere 30 (1995) 2079.
[19] B. Krock, W. Vetter, B. Luckas, Chemosphere 35 (1997) 1519.
[20] S. Ruppe, W. Vetter, B. Luckas, G. Hottinger, J. Microcol. Sep. 12 (2000) 541.
[21] R. von der Recke, E. Mariussen, U. Berger, A. Götsch, D. Herzke, W. Vetter, Organohal. Compd. 66 (2004) 213.
[22] P. Haglund, J. Chromatogr. A 724 (1996) 219.
[23] W. Vetter, Food Rev. Int. 17 (2001) 113.