Enantioselective separation of atropisomeric PBB 132 and PBB 149 in extracts from a Norwegian white-tailed sea eagle egg

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Introduction

Technical mixtures of polybrominated biphenyls (PBB) have been extensively used as flame-retardants in textile and electronic industries, and as additives in plastics[1]. Despite a continuous reduction of the worldwide annual production in the last decades, the presence of PBBs in the environment was recently confirmed in a wide range of samples[2]. PBBs exist in a theoretical variety of 209 congeners. Under environmental conditions, many PBB congeners containing two to four *ortho*-bromine substituents form stable atropisomers. The enantiomer separation of atropisomeric PBBs isolated from a technical mixture was recently published[3]. The purpose of this work was to study the enantioselective fate of environmentally relevant PBBs in egg extracts from Norwegian birds of prey.

Materials and Methods

Egg sample. 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).

Chemicals. Technical hexabromobiphenyl (Firemaster BP-6®, Michigan Chemicals) was used for identification of single PBB congeners as described elsewhere[3]. Solvents, reagents and gasses were of best commercially available quality.

Extraction and clean-up. Cold column extraction of approximately 20 g of the white-tailed sea eagle egg and clean-up of the extract by gel permeation chromatography and on a florisil column were performed as described elsewhere[4].

Group separation. Organobromines were separated from PCBs by group separation on 8 g of activated silica as described in the literatrure[5]. The method was adapted to our laboratory and slightly modified in terms of fraction volumes.

HPLC fractionation. To separate the PBBs from PBDEs and remaining organochlorine pesticides the organobromine containing extract from the group separation was further fractionated on two serially coupled silica HPLC columns. Fraction volumes of 1 to 1.5 mL were collected applying an isocratic flow of 1 mL/min *n*-hexane. These fractions were concentrated for GC-MS analysis.

Enantioselective HPLC/UV. For separation of the atropisomers of PBB 132 an enantioselective HPLC-system similar to the one described in the literature was used[3]. The separation was performed at room temperature on a column coated with

heptakis(2,3,6-tri-*O*-methyl)-ß-cyclodextrin on silica (ß-PMCD, Macheray-Nagel) employing an isocratic flow of 0.5 mL/min acetonitrile/water (60:40, v/v). Fractions were collected as shown in Figure 1A. The fractions were spiked with $^{13}C_{12}$-PBDE 77 as extraction performance standard and extracted five times with *n*-hexane. These extracts were concentrated and octachloronaphthalene (OCN) was added as volumetric standard for non-chiral GC/MS analysis.

Non-chiral GC/MS. The extracts from enantioselective HPLC were analyzed on a Mega II 8065 gas chromatograph (Fisons) coupled to an MD800 quadrupole mass spectrometer (Finnigan) operated in the electron ionization mode (GC/EI-MS) as described elsewhere[3]. Samples were injected on-column onto a DB5MS capillary column (J&W Scientific). The mass spectrometer was operated in selected ion monitoring mode (SIM), using the six characteristic ions m/z 625.5, 627.5 (from $[M]^+$ of hexabromobiphenyls), 495.8, 497.8 (from $[M]^+$ of $^{13}C_{12}$-PBDE 77), 403.8, 405.8 (from $[M]^+$ of OCN).

Enantioselective GC/MS-MS. Enantioselective GC/MS-MS measurements were performed with a CP-3800 gas chromatograph coupled to a 1200 triple quadrupole MS (Varian). Enantioseparations were performed on a 20 m x 0.25 mm i.d. capillary column coated with a 0.15 µm film of the chiral stationary phase consisting of 35 % randomly derivatized 6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin diluted in PS086 (β-TBDM). The GC oven program for PBB 149 started at 80 °C (hold 1 min), then at 20 °C/min to 190 °C (hold 93.5 min), and finally at 5 °C/min to 210 °C/min (hold 26 min). Injections were performed in splitless mode. Helium was used as carrier gas at a flow rate of 1.0 mL/min. GC/EI-MS-MS selected reaction monitoring (SRM) experiments were performed with argon as collision gas at a collision cell pressure of ~1 mTorr. Fragmentation of the most abundant $[M]^+$-isotope signal of PBB 149 at m/z 627.6 to the most abundant $[M\text{-}Br]^+$-isotope signal at m/z 546.7 was performed at 20 V collision voltage.

Results and Discussion

PBB contamination of the white-tailed sea eagle egg. As published elsewhere[4], the contamination of Norwegian bird of prey eggs with PBBs is low compared to PCBs or PBDEs. PBB 153 usually is the most prominent congener and its concentration in the analyzed egg was 10 ng/g wet weight. Of the atropisomeric PBBs only PBB 132 and PBB 149 were present in noteworthy concentrations (however <1 ng/g wet weight). Applying the described extraction and fractionation method resulted in total amounts of approximately 12 ng and 3 ng of PBB 132 and 149, respectively, in the final egg extracts.

Group separation and HPLC fractionation. The group separation was optimized with a test mixture containing *p,p'*-DDE, several PCBs, PBDE 77, and Firemaster BP-6® (technical mixture). It was possible to separate most of the PCBs from the other compounds including the PBBs. The silica HPLC was employed to separate the PBBs from the PBDEs and the chlorinated pesticides. This method was optimized with a test mixture containing HCB, β-HCH, heptachlor, *trans*- and *cis*-nonachlor, *p,p'*-DDE, PBDE 77, and Firemaster BP-6®. The two interesting PBB congeners eluted in different fractions and only heptachlor and *p,p'*-DDE partly eluted in the same fractions as PBB 132 and 149.

Enantioseparation of PBB 132 by HPLC. Nine different GC columns coated with chiral stationary phases were tested for the separation of PBB 132 atropisomers, however, none of them was able to separate the enantiomers. Therefore, this was done by enantioselective HPLC and individual collection of the quantitative amounts of the PBB 132 atropisomers. After extraction into *n*-hexane, subsequent quantitative analyses were performed by non-chiral GC/EI-MS. GC/MS quantification was necessary because the low concentration of PBB 132 in the egg and the presence of PBB 153 in the extract prevented direct quantification by HPLC/UV. Figure 1A shows the HPLC enantioseparation of a PBB 132 reference standard and the five fractions finally collected. The HPLC-fractions of the bird egg extract were quantitatively extracted with *n*-hexane, concentrated, and analyzed by GC/EI-MS. Figure 1B shows the GC/MS chromatograms of the five fractions after standardization of the abundance on the y-axis with help of the volumetric standard. PBB 132 was not detected in fraction 1, 3 and 5 (see Figure 1B). Integration of PBB 132 in fractions 2 and 4 resulted in an enantiomeric ratio (ER) of 0.9. This does not allow conclusions about enantiomeric enrichment of PBB 132 in the analyzed egg sample.

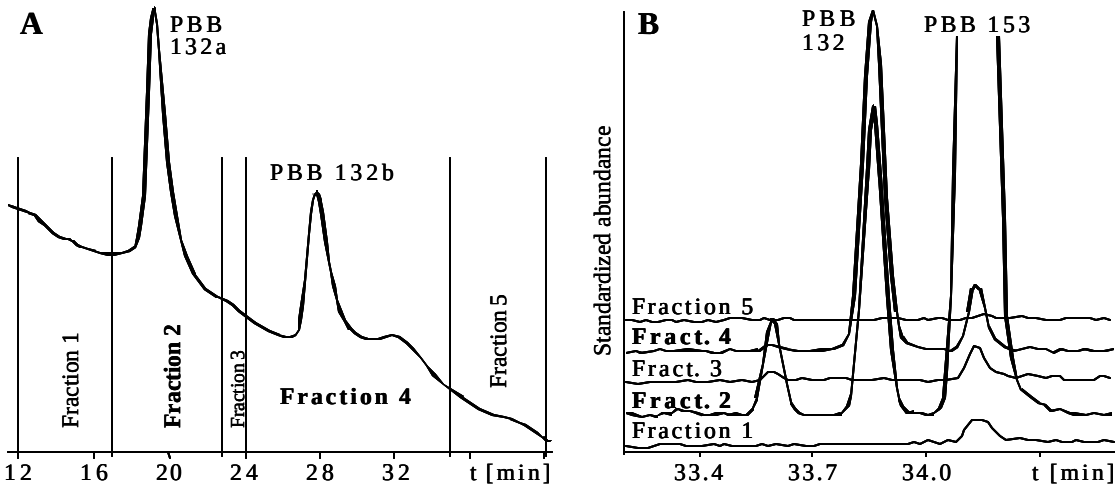

Figure 1. A) HPLC/UV enantioseparation (room temperature) of a PBB 132 reference standard isolated from the technical mixture. The fractions used for collection of single atropisomers from the egg extract are indicated. B) GC/EI-MS chromatograms of the five fractions obtained from chiral HPLC separation of the white-tailed sea eagle egg extract.

Enantioseparation of PBB 149 by GC. Separation of the atropisomers of PBB 149 by enantioselective GC was optimized applying Firemaster BP-6®. It became obvious that a short GC column provided better resolution of the PBB 149 atropisomers due to a lower elution temperature compared to longer columns. However, this positive effect was jeopardized by loss of separation from other brominated compounds. This limited the use of conventional GC/ECNI-MS in the SIM mode when only the bromide ion is recorded (m/z 79 and m/z 81). For this reason, a more selective detection method for PBB 149 had to be developed employing EI-MS-MS. With this technique the racemic composition of PBB 149 in the technical product was confirmed (Figure 2A). The ER for PBB 149

determined in Firemaster BP-6® ranged from 0.97-1.02 (n=4), which confirms the good repeatability of the developed instrumental method. In contrary, enantioselective GC/EI-MS-MS analyses of the bird egg extract confirmed the relative enrichment of the second eluting enantiomer of PBB 149 (Figure 2B). For establishing the ER 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 resulted in ERs of 0.68–0.72 (n=3) for both extracts and thus confirmed the excellent repeatability of the whole method. These findings clearly demonstrate a significant enantioselective enrichment of PBB 149 in a predatory bird egg from Norway, confirming that PBBs can be enantioselectively transformed in higher biota such as birds of prey. It is also noteworthy, that there is a hexabromobiphenyl congener present in the egg extract, eluting just after the PBB 149 atropisomers (Figure 2B), which is not present in the technical mixture (Figure 2A). Such congeners have been observed earlier[4] and could originate from other sources or be degradation products from higher brominated biphenyls.

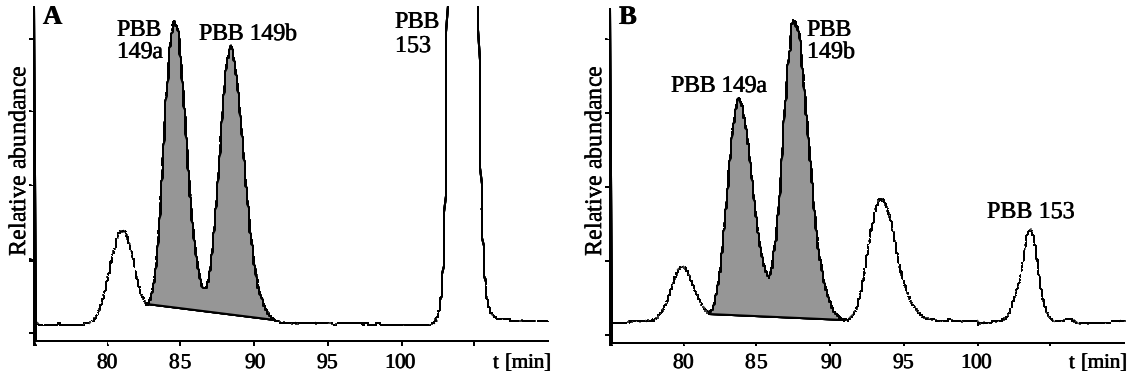

Figure 2. Enantioselective GC/EI-MS-MS separation of PBB 149 in the technical mixture Firemaster BP-6® (A) and in the extract of the white-tailed sea eagle egg (B).

Acknowledgment

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