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 (PBBs) have been extensively used as flame-retardants in textile and electronic industries, and as additives in plastics[1]. Despite a continuous reduction of the production in the last decades, the presence of PBBs in the environment was recently confirmed in a wide range of samples[2]. Under environmental conditions, many PBB congeners 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 the environmentally relevant PBBs 132 (2,2',3,3',4,6'-hexabromobiphenyl) and 149 (2,2',3,4',5',6- hexabromobiphenyl, Figure 1) in egg extracts from Norwegian birds of prey.

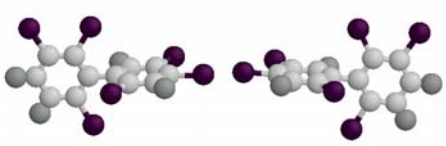

Figure 1. Structures of atropisomers of PBB 149

EXPERIMENTAL

- Egg sample: A non-hatched white-tailed sea eagle egg from Vikna (Norway) collected in 1998. This egg contained 17 ng/g wet weight sum PBBs, whereof 10 ng/g wet weight PBB 153[4]
- Sample preparation: Homogenisation with Na_2SO_4, cold-column extraction, GPC, florisil column, group separation on silica column[5], normal phase HPLC fractionation
- Enantioselective HPLC: Enantioseparation of PBB 132 on a column coated with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin on silica employing a flow of 0.5 mL/min acetonitrile/water (60:40, v/v)[3]. Quantitative analysis of HPLC fractions by non-chiral GC/EI-MS
- Enantioselective GC/EI-MSMS: Enantioseparation of PBB 149 on a column coated with 35 % randomly derivatized 6-*O*-*tert*.-butyl-dimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin in PS086 (β-TBDM)[3]. EI-MSMS detection: m/z 627.6 → m/z 546.7

Sample preparation

Table 1. Separation of brominated compounds from PCBs on 8 g silica

Compound	Fraction 1	Fraction 2	Fraction 3
PCB 138			
PCB 153			
PCB 180			
PBDE 77			
PBB 101			
PBB 132			
PBB 138			
PBB 149			
PBB 153			
PBB 174			
PBB 180			

80 - 100 %
50 - 80 %
20 - 50 %
0 - 20 %
not detected

Fraction 1: 48 mL *n*-hexane
Fraction 2: 10 mL *n*-hexane
Fraction 3: 50 mL *n*-hexane:ethylacetate (10:1)

Table 2. Separation of PBBs from PBDEs and most organochlorine pesticides by normal phase HPLC

Compound	Fract. 1	Fract. 2	Fract. 3	Fract. 4	Fract. 5	Fract. 6
HCB						
p,p'-DDE						
Heptachlor						
β-HCH						
t-NC						
c-NC						
PBDE 77						
PBB 101						
PBB 132						
PBB 138						
PBB 149						
PBB 153						
PBB 174						
PBB 180						

80 - 100 %
50 - 80 %
20 - 50 %
0 - 20 %
not detected

Fract. 1: 3.0 – 7.0 min
Fract. 2: 7.0 – 8.0 min
Fract. 3: 8.0 – 9.5 min
Fract. 4: 9.5 – 10.5 min
Fract. 5: 10.5 – 11.5 min
Fract. 6: 11.5 – 30.0 min

Approximately 12 ng PBB 132 and 3 ng PBB 149 were enriched in two different fractions from 20 g egg sample (see Table 2)

RESULTS AND DISCUSSION

Enantioselective separation of PBB 132 atropisomers

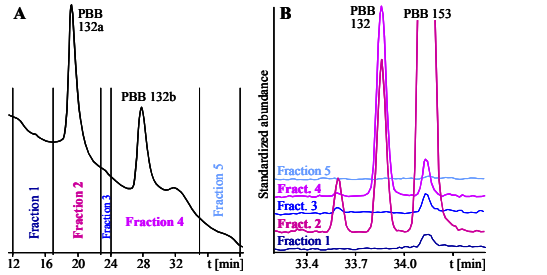

Figure 2. A) HPLC/UV enantioseparation of a PBB 132 reference standard isolated from the technical mixture. B) GC/EI-MS chromatograms of the five fractions obtained from enantioselective HPLC separation of the white-tailed sea eagle egg extract.

Enantiomeric ratio: Bird egg (Figure 2B) 0.92 – 0.99 (n=4)

Enantioselective separation of PBB 149 atropisomers

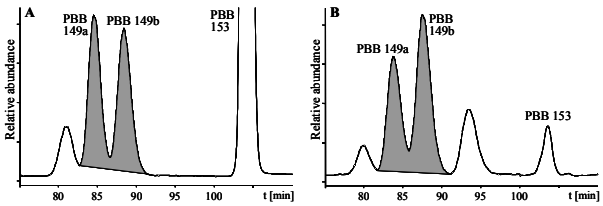

Figure 3. Enantioselective GC/EI-MSMS separation of PBB 149 in the technical mixture Firemaster BP-6® (A) and an extract of the white-tailed sea eagle egg (B).

Enantiomeric ratio: Bird egg (Figure 3B) 0.68 – 0.72 (n=3)

Firemaster® (Fig. 3A) 0.97 – 1.02 (n=4)

CONCLUSIONS

- A very efficient clean-up of the egg sample was developed, leaving mainly PBBs in the extract.
- Atropisomers of PBB 132 could not be separated by enantioselective GC. A combination of enantioselective HPLC and non-chiral GC/MS quantification of the HPLC extracts proved to be successful. Deviation from the racemic mixture could not be found for PBB 132 atropisomers in the white-tailed sea eagle egg.
- Atropisomers of PBB 149 were successfully separated by enantioselective GC/EI-MSMS. An enantiomeric ratio of 0.7 and hence a deviation from the racemic ratio was found in the Norwegian bird of prey egg.

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