Enantiomer-specific Accumulation Pattern of Hexabromocyclododecane in Eggs of Predatory Birds Breeding in Sweden

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Introduction

Hexabromocyclododecane (HBCD) is a widely used additive brominated flame retardant. The technical product consists mainly of a mixture of diastereomers termed a-, ß- and γ-HBCD dominated exclusively by γ-HBCD. HBCD is lipophilic, slowly degradable, accumulates in biota and biomagnifies in the food web with a predominance for the a-isomer[1-3]. All three diastereomers are chiral and exist as enantiomeric pairs. Enantioselective processes might play an important role in adsorption, organ deposition and/or metabolism of pollutants. Enantiospecific accumulation of HBCD has already been confirmed in fish[3]. High levels of total HBCD have recently been reported from Sweden in eggs of guillemot[4] and peregrine falcon[5]. The objectives of this work were:

a) to examine the occurrence of HBCD diastereomers in piscivorous and terrestrial birds.

b) to gain knowledge about enantiomeric ratios of HBCD diastereomers in eggs from birds with different feeding habits.

c) to compare enantiomer fractions for HBCD in guillemot (eggs) with those found in their main food, herring.

Materials and Methods

Eggs from three different bird species were included in this study (Table 1). These were peregrine falcon (*Falco peregrinus)*, a migratory top predator, from two breeding areas in Sweden, white tailed sea eagle (*Haliaeetus albicilla*), a stationary top predator, from two areas in Sweden, and guillemot (*Uria algae*), a piscivorous bird from the Baltic Proper. The northern peregrine population feeds on waders and ducks and the southwestern population on terrestrial bird species. Sea eagle feed on pelagic fish and sea birds, the northern population (Lapland) on species from the fresh water environment, and the other population, from the southern part of the Baltic Sea (Baltic Proper) on species from the marine environment. Sea eagle also feed on carcasses. Guillemot feed mainly on herring. Herring (*Clupea harengus*), from the Baltic Proper as well as from a more northern location (Bothnian Sea) were also analysed. Guillemot eggs and herring were sampled within the Swedish Environmental Monitoring Programme.

Determination of both HBCD diastereomers and enantiomers was performed using LC-MS-MS with a ThermoFinnigan TSQ Quantum MS equipped with a Surveyor HPLC pump as described recently[3]. The MS was used in the electrospray negative ion mode using Selective Reaction Monitoring (SRM) for $[M\text{-}H]^-$ (m/z 640.6) → Br^- (m/z 79.0 and 80.7). Diastereomers were separated by reverse phase chromatography using a Symmetry^{TM} C_{18} (2.1mm x 150mm; 5µm) column (Waters). Chiral chromatography was performed with a ß-cyclodextrin modified silica column (200 x 4.0 mm i.d.) purchased from Macherey-Nagel. A slightly modified mobile phase with 30% H_2O/20% CH_3OH/50% CH_3CN for 0.5 min followed by a linear gradient for 6 min to 45% CH_3OH/55% CH_3CN held for 7 min was used. Extracts were analysed in triplicate except for pooled herring samples (single injection). RSDs are given in Table 1. Standard solutions of a-, ß- and γ-HBCD in toluene were obtained from Cambridge Isotope Laboratories, Inc.

Results and Discussion

Total HBCD levels measured previously in guillemot[4] and peregrine falcon[5] eggs by GC-MS were confirmed by these measurements using LC-MS-MS to be in the range of 80 – 2400 ng/g lipid for falcons and in the range of 54 – 300 ng/g lipids for guillemot. For falcons, higher levels were measured in eggs collected in southwestern Sweden with respect to eggs from the northern population (520 compared to 220 ng/g lipids). Generally, the higher total HBCD levels measured in peregrine falcon eggs might be explained by the fact that peregrine falcon is a top predator, but may also be related to higher exposure from overwintering along the more polluted southern coast of Europe. The guillemot on the other hand, is stationary within the southern Baltic Sea the whole year. Total HBCD levels in sea eagle eggs were estimated only semiquantitatively by LC-MS-MS and the highest values were about 2-5 times higher than levels in peregrine falcon eggs. The diastereomer specific analysis revealed the presence of only α-HBCD in peregrine falcon eggs. Traces of γ-HBCD (<0.1%) were detected in the sea eagle samples with the highest total HBCD levels. ß-HBCD was not found in any of the samples.

The chiral separation of HBCD enantiomers in a standard mixture and in extracts from the different species is shown in Figure 1.

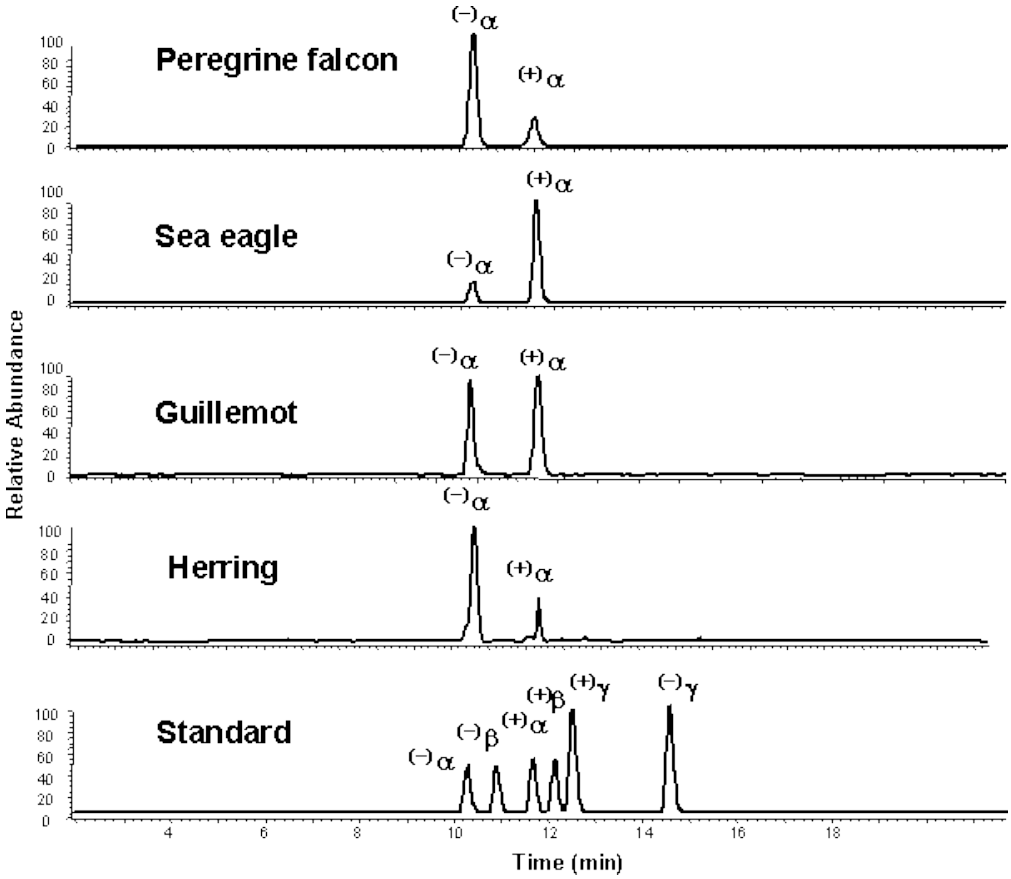

Figure 1. Chromatographic separation of HBCD enantiomers in eggs of peregrine falcon, sea eagle and guillemot, herring muscle and a standard mixture. The elution order of the (+) and (-) enantiomers is according to Heeb *et al*[6].

The enantiomer fractions (EF) were calculated from the peak areas of the enantiomeric pairs using the following formula,

$$\mathbf{EF} = \frac{(+)\mathbf{A}}{(+)\mathbf{A} + (-)\mathbf{A}}$$

in which A is the peak area of the corresponding (+) and (-) enantiomer, respectively. The EFs for α-HBCD in the different species are summarized in Table 1.

Table 1. Sampling data and enantiomer fractions (with corresponding relative standard deviations).

Species	Population	Year	Sampling	EF α	RSD
Falcon *	Northern	1996	1 indiv.	0.33	0.01
Falcon *	Northern	1999	mean, 2 indiv.	0.35	0.02
Mean northern population		1996 - 1999		0.34	0.02
Falcon *	Southwestern	1996	1 indiv.	0.11	0.05
Falcon *	Southwestern	1999	mean, 2 indiv.	0.20	0.04
Mean southwestern population		1996 - 1999		0.17	0.06
Mean of all falcons				**0.21**	**0.12**
Sea Eagle *	Lappland	1996	1 indiv.	0.72	0.09
Sea Eagle *	Lappland	1999	1 indiv.	0.79	0.06
Mean northern (Lapland)		1996 - 1999		0.75	0.08
Sea Eagle *	Baltic Proper	1998	1 indiv.	0.83	0.02
Mean of all sea eagles				**0.77**	**0.07**
Guillemot *	Baltic Proper	1986	pool (10 ind.)	0.60	0.06
Guillemot *	Baltic Proper	1992	pool (10 ind.)	0.59	0.04
Guillemot *	Baltic Proper	1996	1 indiv.	0.51	0.03
Guillemot *	Baltic Proper	1996	1 indiv.	0.50	0.03
Mean of all guillemots				**0.53**	**0.05**
Herring **	Bothnian Sea	1996	pool (5 ind.)	0.25	
Herring **	Baltic Proper	1996	pool (5 ind.)	0.18	
Herring **	Baltic Proper	1999	1 indiv.	0.21	
Herring **	Baltic Proper	1999	pool (5 ind.)	0.31	
Mean of all herrings				**0.25**	**0.07**

* = egg tissue; ** = muscle tissue

The EFs show little variation between different populations of the same species. The exception is the peregrine falcons, where the EF of the northern population is about twice that of the southern population (Table 1). This may reflect the differences in diet, but should be viewed with caution, as it is based on a very small sample size.

Clear differences in α-HBCD EFs were found between the different species analysed (Table 1 and Figure 1). In guillemot eggs, the ratio between the (-) and (+) α-HBCD enantiomers was almost racemic and differs greatly from that found in their feed, Baltic herring. In herring, as in the peregrine falcon, the (-) enantiomer dominates while in sea eagle the (+) enantiomer dominates. This indicates that the enantiomeric pattern in the predator does not necessarily reflect that in the prey. This also indicates that the enantioselective absorption and/or metabolism of α-HBCD vary between different species of birds, resulting in different enantiomeric accumulation. The toxicological significance of these enantiospecific accumulations is so far not known.

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References

1 Morris S., Allchin C.R., Zegers B.N., Haftka J.J.H., Boon J.P., Belpaire C., Leonards P.E.G., van Leeuwen S.P.J., de Boer J. (2004) *Environ. Sci. Technol.* 38: 5497-5504.

2 TomyG.T.,Budakowski W., Halldorson T., Whittle D.M.,KeirM.J.,Marvin C., MacInnis G. (2004) *Environ. Sci. Technol.* 38: 2298-2303.

3 Janák K., Covaci A., Voorspoels S., Becher G. (2005*) Environ. Sci. Technol.* 39: 1987-1994.

4 Sellström U., Bignert A., Kierkegaard A., Häggberg L., de Wit C.A., Olsson M., Jansson B. (2003) *Environ. Sci. Technol.* 37: 5496-5501.

5 Lindberg P., Sellström U., Häggberg L. de Wit C.A. (2004) *Environ. Sci. Technol.* 38: 93-96.

6. Heeb N.V., Schweizer W.B., Kohler M., Gerecke A.C. (2004) *Proceedings of The Third International Workshop on Brominated Flame Retardants* Toronto, Canada, 337-340.