TIME COURSES OF HBCD LEVELS AND ENANTIOMERIC SIGNATURES IN HERRING GULL EGGS FROM THE GERMAN COAST

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Introduction

Hexabromocyclododecane (HBCD) is worldwide one of the most used polybrominated flame retardants and consists of a mixture of three diastereomeric pairs of enantiomers, termed (±) α-, β- and γ-HBCD, with the γ-isomer as main component (α: 1% – 12%, β: 10% – 13%, γ: 75% – 89%)[1-3].In recent years, HBCD has been found widespread in the environment[4-6], leading to a growing awareness of the scientific community especially in the enantiomer-specific bioaccumulation. The investigation of the stereoisomer pattern in biota makes diastereomer- and enantiomer-specific HPLC procedures indispensable.

A growing number of investigations report on the HBCD content in bird eggs from different regions, e.g. for owl eggs in Belgium[7], Baltic Sea bird eggs and muscle[8], gull eggs[9-12] and tawny owls eggs from Norway[13], gull eggs from North America[14,15] and Spitsbergen[16] peregrine falcon eggs from Sweden[17] as well as for miscellaneous bird eggs from South Africa[18] and from Sweden[19]. Most of these studies have used gas chromatographic techniques for the determination of HBCD as sum parameter. So far, only Janak et al.[19] revealed information on the enantiomer-specific behavior of HBCD in bird eggs.

The present study investigated the temporal trends and enantiomeric patterns of HBCD in whole eggs of herring gulls (*Larus argentatus*) collected between 1988 and 2008 from three geographically isolated colonies near the German coast.

Materials and methods

Samples. Eggs of Herring Gulls (*Larus argentatus*) were collected from three uninhabited islands with bird sanctuaries in the North and Baltic Sea. Sampling on the two North Sea islands Trischen (54°3'34" N, 8°41'0" E) and Mellum (53°43'16" N, 8°8'58" E) took place annually from 1988-2008, whereas on Heuwiese (54°25'41" N, 13°7'10" E; Baltic Sea) sampling was done from 1998-2008. At least 35 eggs were collected annually at each sampling site, the whole egg contents were pooled and stored in the German Environmental Specimen Bank (ESB).

For HBCD analysis egg samples originating from 1988, 1991, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008 (North Sea), and 1998, 2000, 2002, 2004, 2006, 2008 (Baltic Sea), respectively, were taken from the archive and freeze-dried.

Chemicals. Native and [$^{13}C_{12}$]-labeled α-, β-, and γ-HBCD standards as racemic solutions in toluene (chemical purity > 98%) were provided by Wellington Laboratories, Inc. (Ontario, Canada). Ammonium acetate, sea sand (washed and ignited), HPLC grade acetonitrile, methanol, dichloromethane, and n-hexane were obtained from J.T. Baker (Deventer, Netherlands). Picograde cyclohexane and ethyl acetate were bought from Promochem (Wesel, Germany). High-purity water was prepared with a Milli-Q system (Millipore, Bedford, USA). SPE cartridges (80 x 15 mm, J.T. Baker, Deventer, Netherlands) were filled with 1 g of activated Florisil (60–100 mesh, Aldrich, Steinheim, Germany, dried and activated at 120 °C for 24 h).

Sample extraction and clean-up. The egg powder was submitted to pressurized fluid extraction on a Dionex ASE™-200 instrument (Dionex Corporation, Sunnyvale, USA). Sample sizes averaged to 1.0 g and were spiked with 50 µL of a 450 ng g^{-1} solution of $^{13}C_{12}$-labeled α-, β- and γ-HBCD resulting in an absolute $^{13}C_{12}$-HBCD content of 4.6 ng for each enantiomer. The void volume of the extraction cell was filled with sea sand (previously baked at 450 °C for 6 h).

Extracts were collected in 60 mL vials and concentrated to 10 mL under a stream of nitrogen. Co-extracted lipids were removed using an automated GPC-system coupled to a UV detector (GPC VARIO, LCTech, Dorfen, Germany) and equipped with an automatic injector and a fraction collector. 6 mL of the sample extract were injected into an S-X3 Bio-Beads gel permeation column (500 mm x 40 mm, L x OD, 50 g of 200 – 400 mesh).

A mixture of cyclohexane/ethyl acetate (1 : 1, v : v) was used as mobile phase with a flow rate of 4 mL min^{-1}. The fraction containing HBCD (80 – 120 mL) was collected in a 100 mL GPC-bottle, evaporated to dryness, re-dissolved in n-hexane and cleaned additionally on 1 g pre-treated Florisil (heated at 120 °C for 24 h) with the following mobile phases: n-hexane (5 mL) and n-hexane/dichloromethane (1 : 1, v : v, 13 mL). Extracts were concentrated to dryness using a gentle stream of nitrogen and re-dissolved in 300 µL of methanol for HPLC-MS/MS analysis. The lipid contents of the egg samples were determined gravimetrically using the not injected portions of the concentrated extracts after GPC clean-up.

HPLC-ESI(-)-MS/MS analysis. Determination of HBCD in egg samples was performed on a HPLC–MS/MS system with electrospray negative ionization (ESI^-). In detail, an Agilent 1200 series HPLC binary pump system (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, an autosampler and a thermostated column compartment was coupled with an API 4000 Q-Trap® high performance hybrid triple quadrupole/linear ion trap mass spectrometer from Applied Biosystems / MDS SCIEX (Foster City, California / Concord, Ontario, Canada).

The chromatographic separation of the analytes was performed using a combination of a Zorbax XDB-C_{18} (double end-capped, pore size: 80 Å, 1.8 µm particle size, 150 x 4.6 mm, Agilent Technologies, Waldbronn, Germany) and a chiral NUCLEODEX β-PM (pore size: 100 Å, 5 µm particle size, 200 x 4.6 mm, Macherey-Nagel GmbH & Co, Düren, Germany) analytical column. The columns were maintained at 15 °C and the mobile phase composition for the separation of HBCD isomers was set to a mixture of 10 mM ammonium acetate buffer (A) and acetonitrile : methanol (90 : 10, v : v) (B) at a flow rate of 600 µL min^{-1}. The elution program started with an initial composition of 15% A and was ramped to 12% A in 3 min. Thereafter it was held for 19 min, followed by an equilibration time (8 min) to return to starting conditions for the next run. The injection volume amounted to 10 µL. The HPLC–MS/MS run time was 30 min per sample, applying a scan width of 1.0 mass units. For the single reaction monitoring mode (SRM), the dwell time was 150 ms per transition. The SRM transitions monitored for native HBCD were 640.6 → 79.0 (quantifier), 640.6 → 81.0 (qualifier) and 652.6 → 79.0 for the $^{13}C_{12}$ labeled HBCD, respectively. MS/MS parameters for each monitored transition were optimized using flow injection analysis. The first and third quadrupoles were set to unit resolution. Source parameters and used MS-software are described elsewhere[20].

Calculation of enantiomer fractions. Enantiomer fractions (EFs) represent the chiral signature and were calculated as follows[21]:

$$EF = \frac{(+)\,E}{(+)\,E + (-)\,E}$$

where (+) E and (-) E represent the peak areas corresponding to (+) and (-) enantiomers. Racemic mixtures show an EF of 0.5, whereas EFs of 0 or 1 indicate pure single enantiomers of (-) or (+), respectively.

Results and discussion

Levels of HBCD stereoisomers. (±)-α-HBCD was detected in all 26 samples in concentrations ranging from 2.06 to 65.9 ng g^{-1} lipid weight (lw) for (-)-α-HBCD and 1.68 - 44.7 ng g^{-1} lw for (+)-α-HBCD. The highest α-HBCD contents were observed in samples from Mellum collected in the year 2000. In all cases α-HBCD represented the dominant diastereomer, whereas β- and γ-HBCD played only a subordinate role. This finding goes along with other reports of HBCD in biota especially in bird eggs[19,22].

Regarding the time trends of (-)- and (+)-α-HBCD contents in eggs from the two North Sea islands Trischen and Mellum (figure 1), it is obvious that courses show similar characteristics. Two distinct stages in development of HBCD levels appear for both series: a first stage when the HBCD content in eggs is relatively low (12/22 ng g^{-1} lw, Trischen/Mellum) and began to rise during the period 1988/1991 – 2000, and a second stage with decreasing HBCD levels after 2000. Eggs from both islands show the lowest contents in 1994. The time course of the (±)-α-HBCD contents in herring gull eggs from the Baltic Sea island Heuwiese is similar apart from the fact that samples prior to the year 1998 were not available. Therefore, the common minimum could not be detected. Nevertheless, the maximum of contents was also determined for the year 2000, followed by an ongoing decrease. The described increases in HBCD contents between 1994 and 2000 are probably due to an increasing demand of HBCD, although it is difficult to interpret details due to scarce information on industrial production and usage.

The determined contents of total HBCD varied between 13.8 – 74.8 ng g^{-1} lw (Trischen), 4.17 – 107 ng g^{-1} lw (Mellum), and 25.1 – 98.7 ng g^{-1} lw (Heuwiese), respectively. Thus, data are within the ranges reported for herring gull eggs sampled in Northern Norway in the years 1983, 1993, and 2003[23]. A significant decrease of HBCD levels in bird eggs after a distinct peak is to the best of our knowledge revealed here for the first time. There are no obvious reasons for the observed decrease of HBCD levels in herring gull eggs on the three German islands during the last years. The enantiomer fractions for α-HBCD are assembled in table 1. The data show a significant deviation from the racemic mixture (EF = 0.5), with the exception of herring gull eggs from Heuwiese in 2002-2008. Heuwiese features the most stable EF_α values and is significantly different from Mellum and Trischen. Only Mellum displays a significant trend towards lower EF_α values. The EF_α reveal throughout an enantiomer-specific enrichment towards (-)-α-HBCD.

A similar accumulation of (-)-α-HBCD was observed in eggs from common tern and peregrine falcon[19]. Here, the EF_α values ranged between 0.14 and 0.34, whereas the EF_α values from sea eagle eggs show an enantiomer-specific enrichment towards (+)-α-HBCD. This result indicates differences in the HBCD source and/or enrichment behavior among the two species. Available data from other biota have shown an enrichment of the first eluting (-)-α-HBCD in several kinds of fish tissues like Swedish herring (EF_α: 0.23-0.24[19]), sole liver and muscle tissue (EF_α: 0.42 and 0.43[24]), and various North Atlantic fish (EF_α: 0.36 – 0.52[25]).

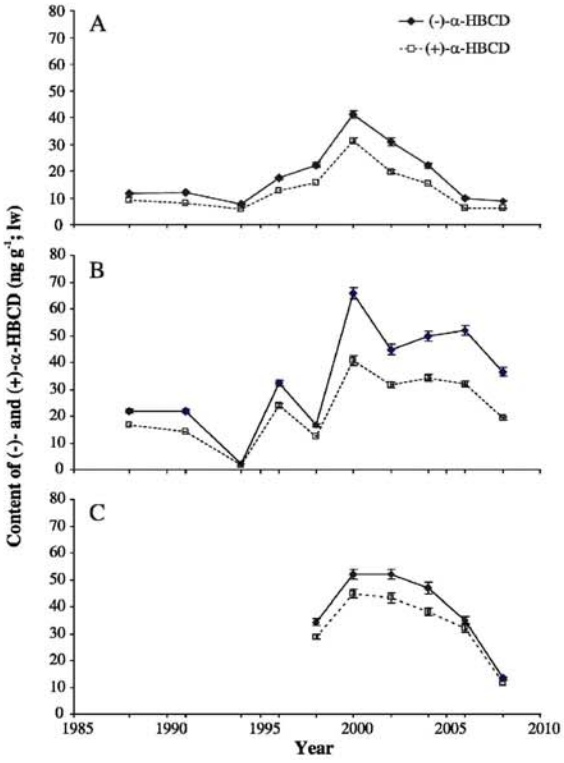

Fig.: 1: Temporal trend of (-)- and (+)-α-HBCD contents (ng g^{-1}; lw) from the three islands Trischen (**A**), Mellum (**B**) and Heuwiese (**C**).Bars on the values display expanded uncertainties (U).

Table 1: EF_α values of α-HBCD in eggs of herring gulls from different islands.

Location	Trischen		Mellum		Heuwiese	
Year	**EF_α value ± U[a]**	**n[b]**	**EF_α value ± U[a]**	**n[b]**	**EF_α value ± U[a]**	**n[b]**
1988	0.44 ± 0.04	120	0.44 ± 0.02	120	-	-
1991	0.39 ± 0.02	140	0.39 ± 0.02	128	-	-
1994	0.43 ± 0.04	120	0.38 ± 0.02	120	-	-
1996	0.43 ± 0.02	120	0.42 ± 0.02	120	-	-
1998	0.41 ± 0.02	125	0.43 ± 0.02	120	0.46 ± 0.03	124
2000	0.43 ± 0.02	120	0.38 ± 0.04	120	0.46 ± 0.03	75
2002	0.39 ± 0.04	110	0.41 ± 0.03	97	0.45 ± 0.04 [c]	50
2004	0.41 ± 0.03	100	0.41 ± 0.02	100	0.45 ± 0.05 [c]	132
2006	0.39 ± 0.05	90	0.38 ± 0.02	90	0.48 ± 0.06[c]	50
2008	0.41 ± 0.05	40	0.34 ± 0.03	50	0.47 ± 0.04 [c]	40

[a] expanded uncertainty (k = 2.8), [b] number of pooled eggs

[c] not significantly different from 0.5; overlap of U(EF_α) and U($EF_{racemate}$)

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