

Hexabromocyclododecane contamination of herring gulls in the coastal area of the southern Baltic Sea

by

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Abstract

Muscles and livers of herring gulls (*Larus argentatus*) found in the coastal area of the southern Baltic Sea were tested for the presence of hexabromocyclododecane (HBCD) isomers. They were detected in the muscles ($\Sigma\text{HBCD} = 42.82 \pm 30.65 \text{ ng g}^{-1} \text{ l.w.}$) and livers ($\Sigma\text{HBCD} = 65.51 \pm 27.96 \text{ ng g}^{-1} \text{ l.w.}$) of all examined gulls. The α -HBCD isomer dominates in all types of samples. Our study has shown that bodies of gulls are less contaminated with HBCD than bodies of aquatic birds from other regions of the world. There was no clear correlation between HBCD concentrations and sex and age of birds. Nevertheless, it was indicated that the highest concentrations of ΣHBCD and the α -HBCD isomer were found in the livers of immature females (mean = $89.31 \pm 21.63 \text{ ng g}^{-1} \text{ l.w.}$ and mean = $76.72 \pm 24.54 \text{ ng g}^{-1} \text{ l.w.}$, respectively). The highest liver sequestration rates of the α -HBCD isomer were found in both adult and immature males (mean = 7.7 ± 13.7 and mean = 6.2 ± 11.9 , respectively).

Key words: HBCD isomers, *Larus argentatus* muscles and liver, Baltic Sea

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Introduction

Hexabromocyclododecane (HBCD) is a bromo-organic compound belonging to the group of polybrominated diphenyl ethers (PBDE) and its presence in the environment is closely related to human activity. HBCD was used in expanded polystyrene (EPS), extruded polystyrene (XPS) and in high impact polystyrene (HIPS), as well as to reduce textile inflammability (de Wit 2002; Alaei et al. 2003; Kajiwara et al. 2009). Its composition is dominated by the γ -HBCD diastereoisomer (about 70%), while α - and β -HBCD constitute from 10 to 30%, and other isomers do not exceed 1% (Heeb et al. 2005). In accordance with the Stockholm Convention on Persistent Organic Pollutants, the use of HBCD is conditionally admissible. Pursuant to Regulation (EC) No. 1907/2006 (REACH), HBCD has been included on the candidate list of Substances of Very High Concern (SVHC) as it is stable and able to accumulate in biota (Covaci et al. 2006; Wu et al. 2011). In biological samples, including humans, the α -HBCD isomer dominates and is characterized by high persistence in the environment (Covaci et al. 2006; Kuiper et al. 2007; Janák et al. 2008). This environmental pollutant can be transported over long distances in the atmosphere and is resistant to photo- and chemical degradation (Remberger et al. 2004; Schure et al. 2004; Law et al. 2008). HBCD affects the thyroid hormone receptor-mediated gene expression and is a potential endocrine disruptor (Janák et al. 2008; Kakimoto et al. 2008; de Wit et al. 2010). HBCD can cause skin sensitization and inhibition of plasma membrane uptake of the neurotransmitters dopamine, glutamate and γ -amino-n-butyric-acid (GABA). Furthermore, it may act as a peroxisome proliferator, which is a mechanism involved in non-mutagenic cancer (Sellström et al. 2003).

Sea birds are an early warning of environmental pollutants and the contamination of their habitat may cause unexpected changes in their numbers, health or breeding success. These animals are commonly used as sentinel organisms (Furness 1993; Grove et al. 2009; Van den Stern et al. 2009) and their eggs are often used as a noninvasive tool for environmental pollution monitoring (e.g. Bignert et al. 2016; Miller et al. 2014; Rigét et al. 2016).

Previous studies on aquatic birds from the Baltic Sea region provide information on its contamination with several persistent organic pollutants (e.g. Falandysz 1984; 1986; Falandysz & Szefer 1983; Falandysz et al. 1994a; 2000; Falkowska et al. 2016; Fliedner et al. 2012; Kannan et al. 2003; Lundstedt-Enkel et al. 2006; Reindl et al. 2019; Thyen et al. 2000). Many researchers also confirm the contamination of bird

habitats with HBCD isomers, even in areas where they have never been produced or used (Braune et al. 2007; Evenset et al. 2007; Jörundsdóttir et al. 2013). HBCD isomers were found in bird organisms and eggs in different regions of the world (e.g. Chen et al. 2012; Elliott et al. 2005; Herzke et al. 2005; Fernie et al. 2009; Marteinson et al. 2012), including the Baltic Sea (Sellström et al. 2003; Lundstedt-Enkel et al. 2006). Our research to date has shown the ability of HBCD to accumulate and accrue in the marine trophic chain, as well as to be transmitted via maternal transfer in *Spheniscus demersus* from the Gdansk Zoo. HBCD isomers were found in the Baltic herring, which represented the only food source for penguins, as well as in the penguin muscles, liver and eggs (Reindl & Falkowska 2015). Further research on eggs of aquatic birds has shown that their contamination depends on the source of food. Eggs of birds feeding on marine food are about 50% more contaminated than eggs of omnivores and scavengers (Reindl & Falkowska 2019).

The liver plays a key role in the internal organ distribution and elimination of several toxins. It also participates in the transformation of environmental pollutants, including HBCD (Abdallah et al. 2014). In birds, the liver is also responsible for the synthesis of egg yolk lipoproteins (Vieira et al. 1995). As part of this process, lipophilic pollutants such as HBCD can be transported internally and deposited in egg lipids. With this in mind, the presented study is focused on the determination of the concentration of HBCD isomers and the selective sequestration factor in the livers of herring gulls collected from the southern coast of the Baltic Sea.

Materials and methods

Biological material for analysis

The herring gull (*Larus argentatus*) is the most common seagull species found on the Polish coast in winter. The species is commonly found in the northern and western parts of Europe. These birds inhabit coastal areas, estuaries and islands, but also inland basins, not shying away from anthropogenic areas. Herring gulls from the Polish coast, especially adult specimens, are sedentary and juveniles most frequently migrate to the west. In winter, individuals from Scandinavia come to the Polish coast. Herring gulls feed on organisms from various trophic levels and their marine diet, which includes fish, invertebrates, crustaceans, small amphibians, eggs and nestlings of other gulls, is often supplemented with carrion and municipal waste (Meissner & Betleja 2007).

The research was carried out on dead herring gulls ($n = 37$), including mature ($n = 7$) and immature ($n = 11$) males and mature ($n = 9$) and immature ($n = 10$) females, collected in accordance with permission of the General Director of Environmental Protection, No. DOPozg1z-4200/III1-169/2015//10/km and of the Regional Director of Environmental Protection in Gdańsk, No. RDOŚ-22-PN.II-6631-4-42/2010/ek.

Dead herring gulls were collected from February 2010 to March 2012 along the southern coast of the Baltic Sea: in winter near the fishing port in Władysławowo ($\varphi = 54^{\circ}47'$, $\lambda = 18^{\circ}25'$) and during the breeding period in the port of Gdynia ($\varphi = 54^{\circ}30'$; $\lambda = 18^{\circ}33'$) and on the beach in Gdansk ($\varphi = 54^{\circ}22'$, $\lambda = 18^{\circ}33'$). After the breeding period, dead herring gulls were found in the "Mewia Lacha" nature reserve situated at the Vistula mouth (Vistula Estuary) and in a small fishing port in Swibno ($\varphi = 54^{\circ}21'$, $\lambda = 18^{\circ}57'$). The causes of their death were not investigated.

The age of birds was determined based on the plumage. Two categories were distinguished: mature/adult – specimens in their 4th and 5th winter coat; immature – specimens in their 1st, 2nd and 3rd coat. The sex of all birds was determined based on genetic tests by DNA amplification using the PCR method described in detail by Fridolfsson & Ellegren (1999).

Reagents and standards

The α -, β - and γ -HBCD isomer standards used in the study were supplied by AccuStandard (New Haven, USA). The extraction solvents, n-hexane and acetone (analytical grade ≥ 99.0 and $\geq 99.9\%$, respectively), used in the chromatographic analysis were supplied by Merck (Warsaw, Poland). Other materials used were sulfuric acid (minimum 95%) and nitric acid (65%), both ultra-analytical grade, supplied by POCH (Gliwice, Poland), acetonitrile hypergrade for LC-MS LiChrosolv, ultrapure water (Merck, Warsaw, Poland), solid-phase extraction (SPE) columns with magnesium silicate (LC-Florisil; Thermo Fisher Scientific, Waltham, Massachusetts, USA), cellulose extraction thimbles (Whatman, Mazidstone, UK) and 99.994% pure nitrogen (Linde, Guildford, UK).

Quality control and assurance

During the analysis, vessels and containers which came into contact with the analyzed organic samples were thoroughly washed and etched in 65% HNO_3 (El-Mekkawi et al. 2009). HBCD was recovered by adding standard concentrations of α -, β - and γ -HBCD isomers separately to the biological sample material before its extraction. The analytical method used

made it possible to recover between 88.5–91.8%, 91.5–94.3% and 79.7–92.6% for α -, β - and γ -HBCD isomers, respectively. In the analytical procedure, tests were carried out on blank samples. The quantification limit (MQL) for the studied compounds was: 0.57 ng cm^{-3} , 0.42 ng cm^{-3} and 0.77 ng cm^{-3} for α -, β - and γ -HBCD isomers, respectively. The results were authenticated by performing parallel tests on selected samples in an accredited laboratory. Comparative tests proved that there were no statistically significant differences between the obtained results.

Sample preparation and extraction

Extraction was carried out using the Soxhlet method with a mixture of n-hexane and acetone in the ratio 1:1. The extract was purified in a reaction with concentrated H_2SO_4 in order to remove lipids, and the final purification was conducted using the solid-phase extraction (SPE) method on column C18 (Remberger et al. 2004). Elution was performed with the solvent used for extraction.

Chemical analyses

HBCD determination was conducted using the method of high performance liquid chromatography (HPLC), in accordance with the methodology described elsewhere (Vilaplana et al. 2009; Köppen et al. 2008) – on the Agilent 1200 series apparatus equipped with a diode array detector (DAD), and run at a fixed wavelength of 208 nm. The separation was carried out on a combined chromatography column: Zorbax Eclipse XDB C18 (Agilent) and chiral NUCLEODEX β -PM. A high purity aqueous solution of acetonitrile (8:2 ratio) was used as a mobile phase in the chromatographic analysis, with a flow rate of 1 ml min^{-1} .

Stable isotope analysis

The analysis of stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in muscles and feathers of gulls was carried out using the Sercon 20–22 Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS) coupled with the Sercon SL elemental analyzer for simultaneous nitrogen-carbon-sulfur (NCS) analysis. Details of the methodology and the calculation method are presented in the paper by Grajewska et al. (2015).

Statistical analysis

The Shapiro–Wilk test was used to assess the normality of distribution of HBCD isomers in the analyzed samples. The significance of differences

was assessed using tests for dependent variables – the t-student test was used for data with parametric distribution and the Wilcoxon test was used for non-parametric variables. The analysis of relationships between variables was carried out based on the Pearson correlation (in the case of parametric data) or the Spearman correlation (non-parametric data). All statistical analyses were performed at a confidence level of 95%. Statistica 10 was used for calculations and visualization of the results.

Results

HBCD isomers were found in the muscles and livers of the examined birds, irrespective of their sexual maturity. Mean Σ HBCD concentrations in herring gull muscles ($42.82 \pm 30.65 \text{ ng g}^{-1} \text{ l.w.}$) were lower than those of Σ HBCD in the liver ($65.51 \pm 27.96 \text{ ng g}^{-1} \text{ l.w.}$). Among the analyzed isomers, the highest concentrations were determined for the α -HBCD isomer, whose contribution to Σ HBCD was on average 80% in both muscles and liver. The correlations were statistically significant and linear ($\alpha\text{-HBCD}_{\text{Muscle}} = 0.4660 \Sigma\text{HBCD}_{\text{Muscle}} + 13.0181$, $p = 0.0000$, $r = 0.7730$; $\alpha\text{-HBCD}_{\text{Liver}} = 0.9175 \Sigma\text{HBCD}_{\text{Liver}} - 7.0279$, $p = 0.0000$, $r = 0.8841$) for both tissues. The average proportion of β - and γ -HBCD isomers in Σ HBCD was about 20%.

In terms of sexual maturity of birds, the concentrations of Σ HBCD in the muscles of mature males were lower than in mature females, while the concentrations of Σ HBCD and α -HBCD in the liver of males were higher than in females. However, the sex of mature herring gulls had no statistically significant effect on the differences in the concentrations of

the studied contaminants (Table 1). A statistically significant effect of sex on varying concentrations of β -HBCD was manifested only in the liver of immature herring gulls ($p = 0.014$). In addition, in this age group, the highest concentrations of the Σ HBCD product and of the α -HBCD isomer were found in females (Table 1). The student's t-test showed significant differences in the concentrations of Σ HBCD ($p = 0.0005$) and α -HBCD ($p = 0.005$) between the muscles and the liver of immature birds.

The HBCD selective hepatic sequestration ratio calculated as the concentration of the assayed parameters in the liver to the concentration in muscles was determined in herring gulls in both sexually mature and immature individuals of both sexes. The highest sequestration rate was found for the α -HBCD isomer in adult and sexually immature males (mean = 7.75 ± 13.70 and mean = 6.18 ± 11.95 , respectively), while the lowest in females (Fig. 1).

Discussion

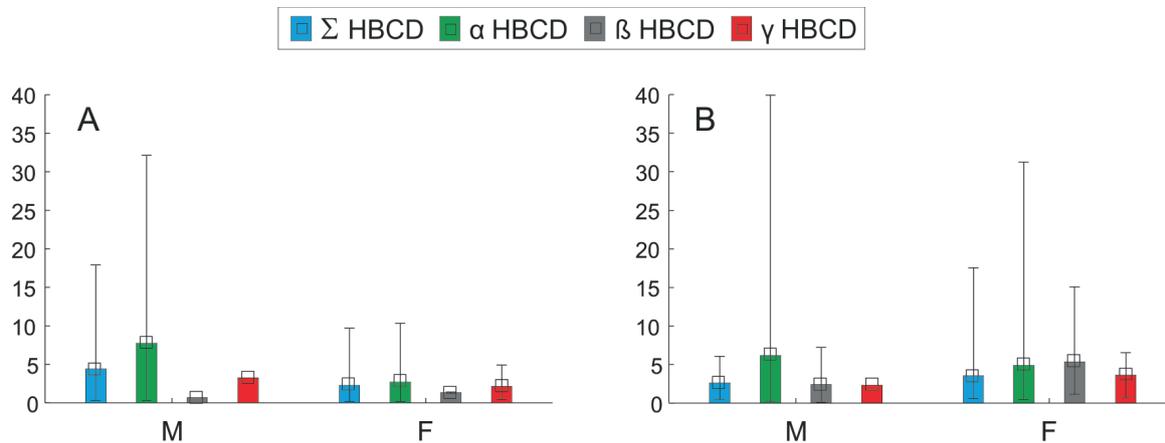
The presence of HBCD in the environment is closely related to anthropogenic activity as it has been used on a significant scale throughout Europe (Remberger et al. 2004). It enters the sea with industrial wastewater and via atmospheric deposition (Schure et al. 2004; Covaci et al. 2006; Feng et al. 2012) and its presence has been confirmed by studies of bottom sediments of rivers entering the Baltic Sea (Sellström et al. 1998). HBCD was found in red algae (*Ceramium tenuicorne*) and in blue mussels (*Mytilus edulis*) from the Baltic Sea (Malmvärn et al. 2005; Bignert et al. 2011). Bignert et al. (2011) indicated temporal and spatial changes in HBCD concentrations in herring (*Clupea harengus*)

Table 1

Statistical characteristics of the concentration of HBCD main isomers and Σ HBCD (in ng per g lipid weight) in the muscles and liver of mature and immature *Larus argentatus* from the Gulf of Gdansk depending on sex

Parameter	Muscles				Liver			
	Male		Female		Male		Female	
	Mature n = 7	Immature n = 11	Mature n = 9	Immature n = 10	Mature n = 7	Immature n = 11	Mature n = 9	Immature n = 10
Lipid (%)	3.23 ± 0.24 (3.01–3.48)	3.23 ± 0.31 (2.76–3.55)	3.19 ± 0.29 (2.85–3.55)	3.43 ± 0.39 (3.17–3.87)	4.51 ± 0.33 (4.23–4.87)	4.24 ± 0.09 (3.98–4.34)	4.92 ± 0.35 (3.50–4.54)	4.46 ± 0.29 (4.13–4.70)
HBCD α -isomer	22.71 ± 19.97 (2.12–55.35)	26.33 ± 21.17 (1.55–71.25)	27.82 ± 17.34 (2.70–51.25)	49.84 ± 28.65 (2.15–87.71)	42.08 ± 24.60 (12.15–71.25)	52.85 ± 23.42 (14.50–85.80)	36.97 ± 21.05 (5.36–65.70)	76.72 ± 24.54 (33.00–112.87)
HBCD β -isomer	3.41 ± 2.48 (1.27–6.12)	5.52 ± 4.95 (0.85–13.32)	7.34 ± 2.60 (4.36–9.15)	3.46 ± 2.75 (0.81–8.71)	4.52 ± 2.65 (2.70–9.14)	3.47 ± 1.98 (1.25–6.14)	7.72 ± 4.38 (2.25–13.02)	6.69 ± 3.89 (1.70–12.22)
HBCD γ -isomer	1.69 ± 0.42 (1.21–1.97)	1.67 ± 0.37 (1.40–1.93)	4.26 ± 2.73 (1.02–7.15)	1.34 ± 1.13 (0.41–2.78)	2.44 ± 1.16 (1.47–3.97)	3.87 ± 2.68 (1.74–7.78)	4.56 ± 1.18 (3.15–5.90)	7.01 ± 4.67 (1.98–11.20)
HBCD Σ isomers *	31.33 ± 29.99 (2.89–83.40)	34.08 ± 18.47 (2.12–73.04)	44.44 ± 42.09 (7.35–146.80)	54.15 ± 28.79 (3.95–92.44)	55.17 ± 31.67 (18.12–91.70)	61.20 ± 21.80 (22.28–95.75)	48.13 ± 22.78 (6.18–81.25)	89.31 ± 21.63 (49.19–123.47)

* Σ HBCD mixture of isomers in the technical product contains a mixture of α -, β -, γ -, ϵ -, δ - isomers

**Figure 1**

HBCD main isomers and the Σ HBCD liver sequestration factor in males (M) and females (F) of mature (A) and immature (B) herring gulls from the coastal area of the southern Baltic

and blue mussels, with a general conclusion on decreasing trends in fish. Janák et al. (2008) reported HBCD in herring at the level of 26 ng g⁻¹ l.w., while the average concentration in herring caught from the southern Baltic Proper was 30 ng g⁻¹ l.w. (Korpinen et al. 2010). Remberger et al. (2004) found HBCD (10–20 ng g⁻¹ l.w.) in muscles of herring caught in 2001 in the Gulf of Bothnia. Reindl and Falkowska (2015) reported a relatively low HBCD concentration in muscles of herring caught in the southern Baltic Sea in 2009–2010 (average: 11.68 ± 4.32 ng g⁻¹ l.w.). The same study carried out on penguins from the Gdansk Zoo, feeding on fish from the southern Baltic, showed the presence of Σ HBCD in penguin muscles at the level of 79.9 ± 15.8 ng g⁻¹ l.w. and indicated the diet as the main route of exposure to environmental pollution. The contamination of penguin bodies was similar to other reports on Baltic birds. The concentration of Σ HBCD detected in the muscles of common guillemots (*Uria aalge*) from a colony on Gotland in the Baltic Sea was at the level of 64.7 ng g⁻¹ l.w. (Lundstedt-Enkel et al. 2006). The present study indicates that the contamination of herring gull muscles with Σ HBCD is at a significantly lower level (42.82 ng g⁻¹ l.w.) than that indicated by previous studies. This shows a significant effect of diet on bird muscle contamination and may also suggest a decreasing trend for these contaminants in the Baltic Sea environment.

This research complements the current knowledge about the HBCD concentration in birds from the *Laridae* family inhabiting the southern Baltic coastal area (Table 2). Studies of the muscles and livers of the herring gull prove the presence of HBCD assayed as a mixture of isomers of the technical product, as

well as the three main isomers of its composition (α -, β - and γ -HBCD), of which the α -HBCD isomer was predominant. Seagulls from the southern Baltic coastal area supplement their marine diet with anthropogenic food (Meissner & Betleja 2007; Szumiło-Pilarska et al. 2017), which would indicate that the diet of marine origin is richer in HBCD than the food available in landfills. The reason for such findings may be the possibility of HBCD accumulation in the trophic chain. HBCD enters the Baltic Sea with sewage and as a result of wet and dry deposition of pollutants emitted into the air. These pollutants are partially accumulated in the sediment and, as a dissolved form, accumulate in fish that are part of seagulls' diet.

With the exception of the Baltic Sea region, HBCD has been found in birds' organs worldwide and the results of studies concerning HBCD concentrations in muscles of aquatic birds (Table 2) from regions with varying degrees of contamination indicate the influence of feeding habits and trophic position on contaminant burdens. The highest levels of HBCD were found in the muscles of large birds of prey, such as the sparrowhawk (*Accipiter nisus*) from the UK (de Boer et al. 2003). Concentrations of Σ HBCD in the Chinese pond heron (*Ardeola bacchus*) periodically inhabiting an electronic waste recycling area in the Pearl River delta in China were in a wide range from 460.0 to 5058.0 ng g⁻¹ l.w., with the predominance of the α -HBCD isomer (He et al. 2010). The study of the glaucous gull (*Larus hyperboreus*) from Bjørnøya in the Barents Sea indicated the presence of the α -HBCD isomer in the liver of birds at a low level of 1.1 ± 4.3 ng g⁻¹ l.w. (Sagerup et al. 2009), while livers of cormorants (*Phalacrocoracidae*) from the British Isles contained

Table 2

Stable isotope ratio of nitrogen and carbon in the muscles of herring gulls collected in the coastal zone of the southern Baltic in 2010–2012 ($\delta^{13}\text{C} = 0.6651 \delta^{15}\text{N} - 30.4105$, $r = 0.5287$, $p = 0.042$)

Parameter	<i>Larus argentatus</i>	<i>Uria aalge</i>	<i>Acipiter nisus</i>	<i>Ardeola bacchus</i>	<i>Amurornis phoenicurus</i>	<i>Gallinago gallinago</i>	<i>Gallinago striatus</i>	<i>Streptopelia chinensis</i>	<i>Francoisina pinnatidens</i>
α -HBCD	33.1 (1.6–87.9)	–	–	1995.0 (420.0–5058.0)	25.5 (nd–66.6)	6.9 (nd–169.0)	nd (nd–42.5)	14.2 (nd–243.0)	19.8 (10.0–25.6)
β -HBCD	4.7 (0.8–13.3)	–	–	nd (nd–8.7)	nd (nd–22.9)	nd (nd–27.1)	nd	nd (nd–19.1)	0.3 (nd–4.7)
γ -HBCD	2.4 (0.4–2.0)	–	–	nd (nd–64.0)	27.0 (nd–305.0)	14.1 (nd–27.1)	nd (nd–216.0)	54.3 (16.6–229.0)	22.0 (20.2–75.2)
Σ HBCD	40.6 (2.1–146.8)	64.7 (53.2–78.7)	84–19000	1995.0 (460.0–5058.0)	73.4 (nd–394.0)	52.0 (nd–344.0)	nd–216.0	73.6 (19.0–492.0)	47.5 (30.5–99.7)
Sampling date	2010–2012	2000–2002	1975–2001	2005–2008	2005–2008	2005–2008	2005–2008	2005–2008	2005–2008
Sampling region	Baltic Sea – southern coastal area	Baltic Sea – Stora Karlsö island	United Kingdom	Pearl River, Qingyuan Country, China					
Type of habitat	marine ecosystem, coastal region	marine ecosystem	–	river delta, electronic waste recycling region					
References	this study	Lundstedt-Enkel et al. 2006	De Boer et al. 2004	He et al. 2010					

nd – not detected

796–1200 ng g⁻¹ l.w. (de Boer et al. 2003).

The carbon stable isotopes ($\delta^{13}\text{C}$ reflecting the feeding habitat) and nitrogen ($\delta^{15}\text{N}$ reflecting the trophic level) detected in the muscles of the herring gull increases with the trophic position (Fig. 2). This confirms the influence of food sources from at least two trophic levels on Σ HBCD and α -HBCD concentrations in bird muscles (Σ HBCD = 13.15 $\delta^{15}\text{N} - 94.31$, $r = 0.59$, $p = 0.05$; α -HBCD = 15.11 $\delta^{15}\text{N} - 119.31$, $r = 0.76$, $p = 0.006$). In the liver of gulls, the effect of the stable isotope ratio on the concentration of hexabromocyclododecane was not statistically significant. Other researchers report a similar finding (de Boer et al. 2003; He et al. 2010).

The bird's liver plays an important role in the processes of detoxification, redistribution and transformation of xenobiotics due to its ability to accumulate large amounts of external impurities (Braune & Norstrom 1989; Kubota et al. 2013; Abdallah et al. 2014). In three quarters of the examined gulls, the obtained results indicate a higher HBCD burden on the liver than on the muscle tissue (Fig. 1). It is likely that chronic exposure contributes to the selective accumulation of xenobiotics in the liver. The β -HBCD isomer in mature and immature males was characterized by the lowest degree of retention in the liver (liver/muscle ratio < 1). This suggests an advantage of elimination over sequestration, for example by deposition in guano, as was the case with penguin feeding on Baltic fish (Reindl & Falkowska 2015), or horn-like formations of epidermis (feathers, claws), as observed in the case of mercury by Grajewska et al. (2019).

A high sequestration factor was found for females, which suggests that lipophilic contaminants can be transferred from the mother's liver to the eggs during their formation. As reported by Vieira et al. (1995), the

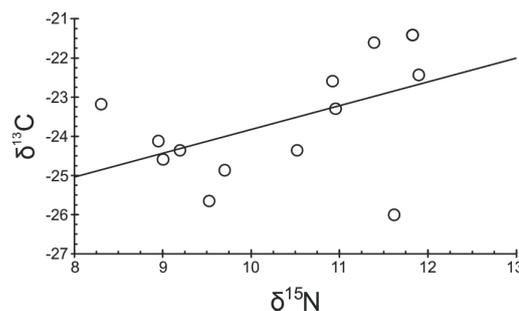


Figure 2

Stable isotope ratio of nitrogen and carbon in the muscles of herring gulls collected in the coastal zone of the southern Baltic in 2010–2012 ($\delta^{13}\text{C} = 0.6651 \delta^{15}\text{N} - 30.4105$, $r = 0.5287$, $p = 0.042$)

liver of birds during egg formation is responsible for lipids included in the egg yolk synthesis. The process of egg formation promotes the transfer of lipophilic pollutants from the liver to the egg, thus burdening the future generation with environmental pollutants (McIndoe 1971). Since xenobiotics are selectively retained in the liver, the transfer to the next generation can also be selective, but the scale of this process should be further researched.

The fact that the effect of sex and age on the HBCD burden in the system of a gull could not be clearly determined is related to its condition and cause of death. Research on dead birds have some limitations and the effect of sex and age may be difficult to assess. It is likely that in emaciated, starved birds, part of HBCD in the muscles and liver was mobilized into the bloodstream due to a hungry body catabolizing its own tissues. The problem of birds' condition, related to the condition of their organisms and the level of xenobiotics in their tissues, has been discussed by scientists (Kalisieńska et al. 2010; Mallory & Braune 2018). This issue requires further research, as there are no clear opinions, especially in the case of wild organisms.

Summary

Herring gulls inhabit the southern Baltic coastal area in quite large numbers, mostly leading a sedentary existence. The abundance of marine and anthropogenic food in the regional environment resulted in a very wide range of concentrations of HBCD and its isomers in the liver and muscles of omnivorous birds. Due to the direct connection between the organism and the environment, these birds can be an excellent indicator of pollution with bromo-organic compounds and serve as an early ecotoxicological warning.

The research conducted on the group of herring gulls from the southern Baltic coast indicated a lower HBCD burden on their organisms than in other birds whose main food is of marine origin.

The authors of the study believe that it is necessary to continue the research along with hepatic sequestration, which affects the level of internal transfer of xenobiotics from the bird's liver to eggs and determines their transfer to the next generation.

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Compliance with Ethical Standards

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Conflict of Interest: All authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Birds used for the analysis were collected according to the permission of the General Director of Environmental Protection (No. DOPozg1z-4200/III-169/2015"/10/km) and of the Regional Director of Environmental Protection in Gdańsk (No. RDOŚ-22-PN.II-6631-4-42/2010/ek). This article does not contain any studies with human participants performed by any of the authors.

References

- Abdallah, M.A.E., Uchea, C., Chipman, J.K. & Harrad, S. (2014). Enantioselective biotransformation of hexabromocyclododecane by in vitro rat and trout hepatic sub-cellular fractions. *Env. Sci. Tech.* 48(5): 2732–2740.
- Alaee, M., Arias, P., Sjödin, A. & Bergman, A. (2003). An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ Int.* 29: 683–689.
- Bignert, A., Berger, U., Boalt, E., Borg, H., Danielsson, S. et al. (2011). Comments Concerning the National Swedish Contaminant Monitoring Programme in Marine Biota. *Report to the Swedish Environmental Protection Agency.*
- Bignert, A., Danielsson, S., Faxneld, S. & Nyberg, E. (2016). *Comments concerning the national Swedish contaminant monitoring programme in marine biota, 2016.* <https://www.diva-portal.org/smash/get/diva2:908725/FULLTEXT01.pdf>
- Braune, B.M., Mallory, M.L., Gilchrist, H.G., Letcher, R.J., & Drouillard, K.G. (2007). Levels and trends of organochlorines and brominated flame retardants in Ivory Gull eggs from the Canadian Arctic, 1976 to 2004. *Sci. Tot. Environ.* 378(3): 403–417.
- Braune, B.M., & Norstrom, R.J. (1989). Dynamics of organochlorine compounds in herring gulls: III. Tissue distribution and bioaccumulation in Lake Ontario gulls. *Environ. Toxicol. Chem.* 8(10): 957–968.
- Chen, D., Letcher, R.J., Burgess, N.M., Champoux, L., Elliott, J.E. et al. (2012). Flame retardants in eggs of four gull species (Laridae) from breeding sites spanning Atlantic to Pacific Canada. *Environ. Poll.* 168: 1–9.
- Covaci, A., Gerecke, A.C., Law, R.J., Voorspoels, S., Kohler, M.

- et al (2006). Hexabromocyclododecane (HBCDD) in the environment and humans: A review. *Environ. Sci. Technol.* 40: 3679–3688.
- de Boer, J., Leonards, P.E., Kwadijk, C.J., Kruijt, A.W., Brandsma, S.H. et al. (2003). Screening and time trend study of decabromodiphenylether in birds (No. C051/03). *RIVO*.
- de Wit, C.A. (2002). An overview of brominated flame retardants in the environment. *Chemosphere* 46: 583–624.
- de Wit, C.A., Herzke, D. & Vorkamp, K. (2010) Brominated flame retardants in the arctic environment – trends and new candidates. *Sci. Tot. Environ.* 408(15): 2885–2918.
- Elliott, J.E., Wilson, L.K. & Wakeford, B. (2005). Polybrominated diphenyl ether trends in eggs of marine and freshwater birds from British Columbia, Canada, 1979–2002. *Environ. Sci. Tech.* 39(15): 5584–5591.
- El-Mekkawi, H., Diab, M., Zaki, M., & Hassan, A. (2009). Determination of chlorinated organic pesticide residues in water, sediments and fish from private fish farms at Abbassa and Sahl Al-Husainia, Sharkia Governorate. *Aust. J. Basic & Appl. Sci.* 3(4): 4376–4383.
- Evenset, A., Carroll, J., Christensen, G.N., Kallenborn, R., Gregor, D. et al. (2007). Seabird guano is an efficient conveyer of persistent organic pollutants (POPs) to Arctic lake ecosystems. *Environ. Sci. Tech.* 41(4): 1173–1179.
- Falandysz, J. (1984). Metals and organochlorines in a female white-tailed eagle from Uznam Island, southwestern Baltic Sea. *Environ. Conserv.* 11(3): 262–263.
- Falandysz, J. (1986). Metals and organochlorines in adult and immature males of white-tailed eagle. *Environ. Conserv.* 13(1): 69–70.
- Falandysz, J. & Szefer, P. (1984). Chlorinated hydrocarbons in fish-eating birds wintering in the Gdańsk Bay, 1981–82 and 1982–83. *Mar. Poll. Bull.* 15(8): 298–301.
- Falandysz, J., Yamashita, N., Tanabe, S., Tatsukawa, R., Rucińska, L. et al. (1994). Congener-specific analysis of polychlorinated biphenyls in white-tailed sea eagles *Haliaeetus albicilla* collected in Poland. *Arch. Environ. Cont. Tox.* 26(1): 13–22.
- Falandysz, J., Strandberg, L., Mizera, T., & Kalisińska, E. (2000). The contamination of white-tailed sea eagles with organochlorines in Poland. *Roczniki PZH.* 51(1): 7–13.
- Falkowska, L., Reindl, A.R., Grajewska, A. & Lewandowska, A.U. (2016). Organochlorine contaminants in the muscle, liver and brain of seabirds (*Larus*) from the coastal area of the Southern Baltic. *Ecotox. Environ. Safe.* 133: 63–72. DOI: 10.1016/j.ecoenv.2016.06.042.
- Feng, A.H., Chen, S.J., Chen, M.Y., He, M.J. & Luo, X.J. (2012). Hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) in riverine and estuarine sediments of the Pearl River Delta in southern China, with emphasis on spatial variability in diastereoisomer- and enantiomer-specific distribution of HBCD. *Mar. Poll. Bull.* 64(5): 919–925.
- Fernie, K.J., Shutt, J.L., Letcher, R.J., Ritchie, I.J. & Bird, D.M. (2009). Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. *Environ. Sci. Tech.* 43(6): 2124–2130.
- Fliedner, A., Rüdell, H., Jüriling, H., Müller, J., Neugebauer, F. et al. (2012). Levels and trends of industrial chemicals (PCBs, PFCs, PBDEs) in archived herring gull eggs from German coastal regions. *Environ. Sci. Eur.* 24(17): 7.1–15 DOI: 10.1186/2190-4715-24-7.
- Fridolfsson, A.K. & Ellegren, H. (1999). A simple and universal method for molecular sexing of non-ratite birds. *J. Avian Biol.* 116–121.
- Furness, R.W. (1993). Birds as monitors of pollutants. In *Birds as monitors of environmental change* (pp. 86–143). Springer.
- Grajewska, A., Falkowska, L., Szumiło-Pilarska, E., Hajdrych, J., Szubska, M. et al. (2015). Mercury in the eggs of aquatic birds from the Gulf of Gdansk and Włocławek Dam (Poland). *Environ. Sci. Pollut. Res.* 22(13): 9889–9898. DOI: 10.1007/s11356-015-4154-y.
- Grajewska, A., Falkowska, L. & Reindl, A. (2019). Evaluation of claws as an alternative route of mercury elimination from the herring gull (*Larus argentatus*). *Oceanol. Hydrobiol. St.* 48(2): 165–173.
- Grove, R.A., Henny, C.J. & Kaiser, J.L. (2009). Osprey: worldwide sentinel species for assessing and monitoring environmental contamination in rivers, lakes, reservoirs, and estuaries. *J. Toxicol. Environ. Health. B.* 12(1): 25–44.
- He, M.J., Luo, X.J., Yu, L.H., Liu, J., Zhang, X.L. et al. (2010). Tetrabromobisphenol-A and hexabromocyclododecane in birds from an e-waste region in South China: influence of diet on diastereoisomer- and enantiomer-specific distribution and trophodynamics. *Environ. Sci. Tech.* 44(15): 5748–5754.
- Heeb, N.V., Schweizer, W.B., Kohler, M., & Gerecke, A.C. (2005). Structure elucidation of hexabromocyclododecanes – a class of compounds with a complex stereochemistry. *Chemosphere* 61(1): 65–73.
- Herzke, D., Berger, U., Kallenborn, R., Nygård, T. & Vetter, W. (2005). Brominated flame retardants and other organobromines in Norwegian predatory bird eggs. *Chemosphere* 61(3): 441–449.
- Janák, K., Covaci, A., Voorspoels, S. & Becher, G. (2005). Hexabromocyclododecane in marine species from the Western Scheldt Estuary: diastereoisomer- and enantiomer-specific accumulation. *Environ. Sci. Tech.* 39(7): 1987–1994.
- Janák, K., Sellström, U., Johansson, A.K., Becher, G., de Wit C.A. et al. (2008). Enantiomer-specific accumulation of hexabromocyclododecane in eggs of predatory birds. *Chemosphere* 73: S193–S200.
- Jörundsdóttir, H., Löfstrand, K., Svavarsson, J., Bignert, A. & Bergman, Å. (2013). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) in seven different marine bird species from Iceland. *Chemosphere*

- 93(8): 1526–1532.
- Kajiwara, N., Sueoka, M., Ohiwa, T. & Takigami, H. (2009). Determination of flame-retardant hexabromocyclododecane diastereomers in textiles. *Chemosphere* 74(11): 1485–1489.
- Kakimoto, K., Akutsu, K., Konishi, Y. & Tanaka, Y. (2008). Time trend of hexabromocyclododecane in the breast milk of Japanese women. *Chemosphere* 71: 1110–1114
- Kalińska, E., Budis, H., Podlasińska, J., Łanocha, N. & Kavetska, K.M. (2010). Body condition and mercury concentration in apparently healthy goosander (*Mergus merganser*) wintering in the Odra estuary, Poland. *Ecotoxicology* 19(8): 1382–1399.
- Kannan, K., Kumar, K.S., Nakata, H., Falandysz, J., Oehme, G., et al. (2003). Polychlorinated Biphenyls, Dibenzo-p-dioxins, Dibenzofurans, and p, p'-DDE in Livers of White-Tailed Sea Eagles from Eastern Germany, 1979–1998. *Environ. Sci. Tech.* 37(7): 1249–1255.
- Korpinen, S. (Ed.), Laamanen, M. (Ed.), Andersen, J.H., Asplund, L., Berger, U. et al. (2010). *Hazardous substances in the Baltic Sea: An integrated thematic assessment of hazardous substances in the Baltic Sea*. (Baltic Sea Environment Proceedings; Vol. 2010, No. 120B). Helsinki, Finland: Helsinki Commission.
- Köppen, R., Becker, R., Jung, C. & Nehls, I. (2008). On the thermally induced isomerisation of hexabromocyclododecane stereoisomers. *Chemosphere* 71(4): 656–662.
- Kubota, A., Yoneda, K., Tanabe, S. & Iwata, H. (2013). Sex differences in the accumulation of chlorinated dioxins in the cormorant (*Phalacrocorax carbo*): implication of hepatic sequestration in the maternal transfer. *Environ. Poll.* 178: 300–305.
- Kuiper, R.V., Canton, R.F., Leonards, P.E.G., Jenssen, B.M., Dubbeldam, M. et al. (2007). Long-term exposure of European flounder (*Platichthys flesus*) to the flame-retardants tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBDC). *Ecotoxicol. Environ. Safe.* 67: 349–360.
- Law, R.J., Allchin, C.R., de Boer, J., Covaci, A., Herzke, D. et al. (2006) Levels and trends of brominated flame retardants in the European environment. *Chemosphere* 64: 187–208.
- Lundstedt-Enkel, K., Asplund, L., Nylund, K., Bignert, A., Tysklind, M. (2006) Multivariate data analysis of organochlorines and brominated flame retardants in Baltic sea guillemot (urja aalge) egg and muscle. *Chemosphere* 65: 1591–1599.
- Mallory, M.L. & Braune, B.M. (2018). Do concentrations in eggs and liver tissue tell the same story of temporal trends of mercury in high Arctic seabirds?. *J. Environ. Sci.* 68: 65–72.
- Malmvärn, A., Marsh, G., Kautsky, L., Athanasiadou, M., Bergman, Å. et al. (2005). Hydroxylated and methoxylated brominated diphenyl ethers in the red algae *Ceramium tenuicorne* and blue mussels from the Baltic Sea. *Environ. Sci. Tech.* 39(9): 2990–2997.
- Martinson, S.C., Bird, D.M., Letcher, R.J., Sullivan, K.M., Ritchie, I.J. et al. (2012). Dietary exposure to technical hexabromocyclododecane (HBDC) alters courtship, incubation and parental behaviors in American kestrels (*Falco sparverius*). *Chemosphere* 89(9): 1077–1083.
- McIndoe, W.M. (1971). Yolk synthesis. In D.J. Bell & B.M. Freeman. *Physiology and biochemistry of the domestic fowl*. London. UK, Academic Press, Inc.
- Meissner, W. & Betleja, J. (2007). Skład gatunkowy, liczebność i struktura wiekowa mew Laridae zimujących na składowiskach odpadów komunalnych w Polsce. *Not. Orn.* 48: 11–27.
- Miller, A., Nyberg, E., Danielsson, S., Faxneld, S., Haglund, P. et al. (2014). Comparing temporal trends of organochlorines in guillemot eggs and Baltic herring: advantages and disadvantage for selecting sentinel species for environmental monitoring. *Mar. Environ. Res.* 100: 38–47. DOI: 10.1016/j.marenvres.2014.02.007.
- Reindl, A.R. & Falkowska, L. (2015). Flame Retardants at the Top of a Simulated Baltic Marine Food Web – A Case Study Concerning African Penguins from the Gdansk Zoo. *Arch. Environ. Cont. Tox.* 68(2): 259–264.
- Reindl, A.R. & Falkowska, L. (2019). Food sources as a factor determining birds' exposure to hazardous organic pollutants and its eggs contamination. *Mar. Freshwater Res.* DOI: 10.1071/MF19159.
- Reindl, A.R., Falkowska, L. & Grajewska, A. (2019). Halogenated organic compounds in the eggs of aquatic birds from the Gulf of Gdansk and Włocławek Dam (Poland). *Chemosphere* 237: 124463.
- Remberger, M., Sternbeck, J., Palm, A., Kaj, L., Strömberg, K. et al. (2004). The environmental occurrence of hexabromocyclododecane in Sweden. *Chemosphere* 54(1): 9–21.
- Rigét, F., Vorkamp, K., Bossi, R., Sonne, C., Letcher, R.J. et al. (2016). Twenty years of monitoring of persistent organic pollutants in Greenland biota. A review. *Environ. Poll.* 217: 114–123.
- Sagerup, K., Helgason, L.B., Polder, A., Strøm, H., Josefsen, T.D. et al. (2009). Persistent organic pollutants and mercury in dead and dying glaucous gulls (*Larus hyperboreus*) at Bjørnøya (Svalbard). *Sci. Tot. Environ.* 407(23): 6009–6016.
- Schure, A.F., Agrell, C., Bokenstrand, A., Sveder, J., Larsson, P. et al. (2004). Polybrominated diphenyl ethers at a solid waste incineration plant II: atmospheric deposition. *Atmospheric Environ.* 38(30): 5149–5155.
- Sellström, U., Kierkegaard, A., de Wit, C. & Jansson, B.O. (1998). Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a Swedish river. *Environ. Tox. Chem.* 17(6): 1065–1072.
- Sellström, U., Bignert, A., Kierkegaard, A., Häggberg, L., de Wit, C.A. et al. (2003). Temporal trends studies on tetra- and pentabrominated diphenyl ethers and hexabromocyclododecane in guillemot egg from Baltic Sea. *Environ. Sci. Technol.* 37: 5496–5501.

- Szumiło-Pilarska, E., Falkowska, L., Grajewska, A. & Meissner, W. (2017). Mercury in feathers and blood of gulls from the Southern Baltic coast, Poland. *Wat. Air Soil Poll.* 228(4): 138.
- Thyen, S., Becker, P.H. & Behmann, H. (2000). Organochlorine and mercury contamination of little terns (*Sterna albifrons*) breeding at the western Baltic Sea, 1978–96. *Environ. Poll.* 108(2): 225–238. DOI: 10.1016/S0269-7491(99)00183-9.
- Wu, J.P., Guan, Y.T., Zhang, Y., Luo, X.J., Zhi, H. et al. (2011). Several current-use, non-PBDE brominated flame retardants are highly bioaccumulative: evidence from field determined bioaccumulation factors. *Environ. Int.* 37(1): 210–215.
- Van den Steen, E., Pinxten, R., Jaspers, V.L., Covaci, A., Barba, E. et al. (2009). Brominated flame retardants and organochlorines in the European environment using great tit eggs as a biomonitoring tool. *Environ. Int.* 35(2): 310–317. DOI: 10.1016/j.envint.2008.08.002.
- Vieira, P.M., Vieira, A.V., Sanders, E.J., Steyrer, E., Nimpf, J. et al. (1995). Chicken yolk contains bona fide high density lipoprotein particles. *J. Lip. Res.* 36(3): 601–610.
- Vilaplana, F., Ribes-Greus, A. & Karlssona, S. (2009). Microwave-assisted extraction for qualitative and quantitative determination of brominated flame retardants in styrenic plastic fractions from waste electrical and electronic equipment (WEEE). *Talanta* 78: 33–39. DOI: 10.1016/j.talanta.2008.10.038.