

## TEMPORAL TRENDS OF CLASSICAL AND EMERGING HALOGENATED FLAME RETARDANT LEVELS IN BIRD EGGS

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### Introduction

Flame retardants (FRs) are compounds added to or reacted with polymeric material with the aim of increasing the fire resistant properties. Polybrominated diphenyl ethers (PBDEs) are the family of 209 different congeners of brominated flame retardants (BRFs) most widely used during last years, but their toxic effects and capability to bioaccumulate in the environment have caused their classification as persistent organic pollutants (POPs) by the Stockholm convention<sup>1</sup>. Even though the toxicity mechanisms of these FRs are not completely known, recent studies have demonstrated that these compounds cause adverse effects on the animal health and their presence in the environment is a matter of great concern. Therefore, their use has been forbidden for the global production and usage in polymeric material and consequently, many chemicals have emerged as substitutes, with similar physicochemical properties, but probably with similar behavior in the nature, as the case of halogenated norbornenes (HNs), such as Dechlorane Plus (DP) and its relatives (Dec-602, -603 and -604). DP has been thought like the replacement for the restricted Deca-BDE.

The objective of this study was to determine temporal trends in concentration levels of banned BFRs as well as emerging BFRs. The occurrence of classical PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209) and emerging FRs (Dec-602, Dec-603, Dec-604 and DP) in bird eggs was determined. Samples are of non-hatched eggs from different species of birds collected at different periods of time in Doñana Natural Park and surrounding areas (South-East of Spain). The bird species chosen are examples of species rich in an aquatic and terrestrial diet: black kites, white storks and greater flamingos.

### Materials and methods

#### Sample collection

108 bird eggs corresponding to three different species and collected in two different periods were analysed. Table 1 showed the sampling details.

Table 1. Bird egg samples analysed, corresponding to different bird species and different time periods.

Species	Scientific name	Archived samples (1999-2003)	Contemporary samples (2011-2013)
White stork	<i>Ciconia ciconia</i>	10	39
Black kite	<i>Milvus migrans</i>	10	29
Greater flamingo	<i>Phoenicopterus roseus</i>	10	10

#### Sample preparation

The sample preparation was proceeded treating the frozen content of the eggs. Homogenization, freeze-drying and trituration were previously done before the spike with the mixture of all the internal labelled standards. Extraction was carried out in a pressurized liquid extractor automatized, using a mixture of n-hexane:dichloromethane (1:1 v/v) at 100°C and 1500-1600 psi. The extract obtained was evaporated with N<sub>2</sub> gas and the remaining fat was dried to constant weight to determine the lipid content, and re-dissolved in n-hexane. The fat was removed by an acidic treatment with concentrated H<sub>2</sub>SO<sub>4</sub> and a final cleanup step was performed

using solid phase extraction (SPE) with neutral alumina cartridges and eluting with a mixture of n-hexane/dichloromethane (1:2,v/v).

#### *Instrumental analysis*

For PBDE analysis, the chromatographic separation was carried out on a gas chromatograph Agilent Technologies 7890A with a column DB-5ms (15m x 0,25mm x 0,1 µm of film thickness). Injector temperature was kept at 300°C and 1µL of samples and standards was injected in the splitless injection mode (1 min.). Helium was used as carrier gas to a constant flow-rate of 1 mL/min. The oven temperature was programmed from 90°C (held for 1 min.) to 310°C at a rate of 10°C/min., and finally to 310°C (held for 13 min.). The analysis of the compounds was completed in an Agilent 7000A GC/MS Triple Quadrupole mass spectrometer, working in electron ionization (EI) mode. Selective reaction monitoring (SRM) has been used as mode of working, monitoring the two most intense transitions.

For BDE-209 determinations, the chromatographic separation was carried out on a gas chromatograph Agilent 7890C with a column DB-5ms (15m x 0,25mm x 0,1 µm of film thickness). The volume of injection was 1µL during 1 minute in splitless mode at an injector temperature of 300°C. Helium was used as carrier gas at a flow-rate of 1 ml/min. The oven temperature program was programmed from 140°C (held for 2 min) to 325°C at a rate of 10°C/min, and then this temperature was held during 10 minutes. The total time for the complete separation and the analysis were 30,5 minutes<sup>2</sup>. The analysis of this compound was completed in an Agilent 5975A Network mass spectrometer, working in negative ion chemical ionization (NICI) using NH<sub>4</sub><sup>+</sup> as reagent gas at a flow-rate of 2,25 mL/min and a source temperature of 250°C. Selected ion monitoring (SIM) was used as mode for monitoring the *m/z* 487 and 489 and *m/z* 497 and 499 for BDE-209 and <sup>13</sup>C-BDE-209 respectively.

Finally, HN analyses were carried out as follows. The chromatographic separation was carried out in a gas chromatograph Agilent Technologies 7890A. The same chromatographic conditions for the other experiments were used in this case too, only changing the oven temperature, which was programmed starting at 80°C (held for 2 minutes) and was ramped at 10°C/min until 300°C (held for 10 minutes<sup>3</sup>). Total time of complete separation and analysis was accomplished in 34 min. The analysis of the compounds was completed in an Agilent 7000A GC/MS Triple Quadrupole mass spectrometer, working in NICI using CH<sub>4</sub><sup>+</sup> as reagent gas with a flow-rate of 2,25 mL/min and at a source temperature of 175°C. Electron energy was set at 200 eV, emission current at 150 eV and collision energies were modified from 1 to 35 eV. SRM was used for monitoring the two most intense transitions of each compound.

Quantification was processed using the isotopic dilution method for each compound with its internal standard. Only in the case of HNs, DP and its relatives were quantified from the labelled *syn*-DP.

## **Results and discussion**

### *Concentration levels*

PBDEs were detected in all the species, with total levels ranging from nq to 311 ng/g lw. The most contaminated specie was the white stork with values ranging between 2.05 and 311 ng/g lw, followed by black kite (between 1.80 and 259 ng/g lw), and finally, by greater flamingo (between nq and 25.9 ng/g lw). The variation in PBDE levels among different species was considerable, but variation was also substantial within species. This fact has been reported in other studies about lyphofilic contaminants and is attributed to differences in diet composition. In birds, factors such as age, body condition and habitat may affect the contaminants accumulated by the female, which are transferred to the egg. This variation could be also explained by the migratory behaviour of some of these species. Moreover, in the case of white storks, additional variation may be further promoted by the fact that part of the population migrates while another part winters in the regional surroundings of Doñana.

Eight PBDE congeners were detected, including BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209. Moreover, BDE-209 was the most abundant BDE congener in all the species. Normally, the PBDE profile in biota samples presented a high contribution of the low-brominated BDEs, such as BDE-47, which present a higher bioaccumulation capacity than the high brominated congeners. This has been

specially found in aquatic food webs, but it is not so evident in terrestrial food webs. Moreover, the predominance of BDE-209 in birds from Doñana National Park was also found by Barón *et al*<sup>1</sup>.

As regards to HNs, Dec-602, Dec-603 and DP were detected, whereas Dec-604 was not detected in any sample. Dechloranes were detected in all the species with total values ranging from nd to 260 ng/g lw for white storks, from 1.44 to 75.9 ng/g lw for black kite, and from 5.24 to 248 ng/g lw for greater flamingo. Similarly to PBDEs, the most contaminated specie was the white storks. However, the second one was greater flamingo which presented higher HN levels than black kite.

DP represented the 38% (mean value) of the total HNs. DP has been the most studied compound of the dechlorane family, but the fact that it might not be the most predominant compound has been pointed out in some other studies<sup>2</sup>. In fact, in our study levels of Dec-602 were the highest. Although it has not been studied in birds, studies on fish samples indicated that Dec-602 might have more bioaccumulation and biomagnification potential than DP<sup>3</sup>.

#### Temporal trends

Since the three commercial mixtures of PBDEs have already been banned in Europe, it is to be expected that their environmental levels will decrease and, consequently, levels of alternative FRs such as HNs will rise. In this study we compared the total concentration levels of both FR families (Figure 1).

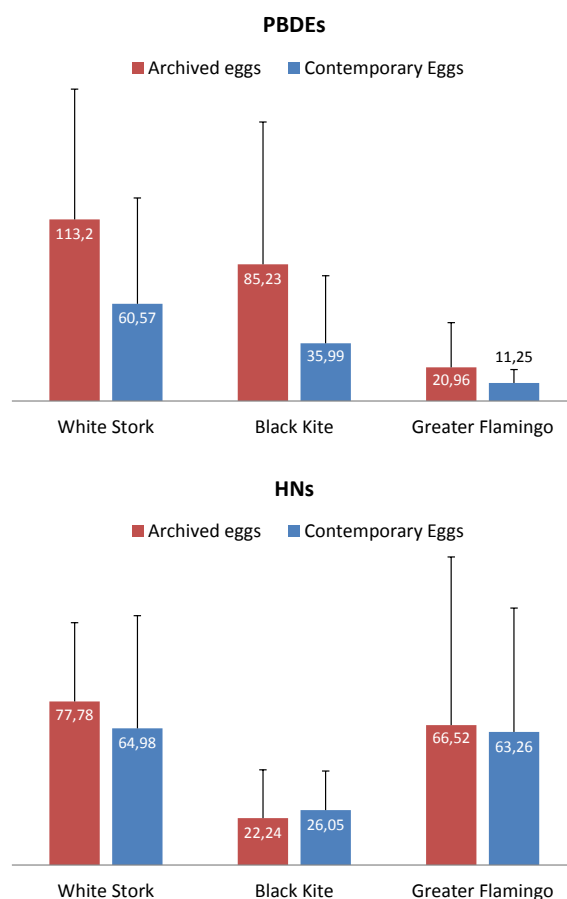


Figure 1. Mean concentration levels (expressed in ng/g lw) of PBDEs and HNs in archived and contemporary bird eggs of white storks, black kites and greater flamingos.

As can be seen, results of total PBDEs showed a decrease of concentration levels with time, with declines ranging from 46% (for white storks and greater flamingos) to 58% (for black kites). If we look separately at the main congeners of Penta-BDE (BDE-47, -99 and -100) and Deca-BDE (BDE-209) mixtures, we note that the decline is more pronounced for the Penta-BDE. For instance, levels of Penta-BDE in white storks decreased from 59.0 to 9.77 ng/g lw (decline of 83%), whereas levels of Deca-BDE decreased from 59.1 to 55.4 ng/g lw (decline of 6%). This would be consistent with the date of prohibition of Penta-BDE mixture that occurred several years earlier than the Deca-BDE.

As regards HNs, concentrations of these FRs were quite similar along the time for the three bird species analysed. These compounds are currently used as FRs, but their occurrence in the environment is not well defined yet. Probably more time is required to observe a clear temporal trend. In any case, an increase of concentration levels reflecting an increased use of these FRs, was not observed.

Levels of HNs were compared with those of PBDEs in each sample analyzed. The ratio  $R = [\Sigma\text{HNs}]/[\Sigma\text{PBDEs}]$  ranged from 0.07 (indicating a higher contamination by PBDEs) to 75.7 (indicating a higher contamination by HNs). If we compare the ratios obtained for archived and contemporary samples, a temporal trend was also observed. In archived white storks, the mean value of R was 0.85. However, for contemporary samples, mean value of R was 2.24, indicating a shift towards a greater use of HNs and/or a lesser use of PBDEs. The same behavior was observed for black kites (mean values of R of 1.55 and 4.97, for archived and contemporary samples) and greater flamingos (mean values of R of 3.75 and 13.9, for archived and contemporary samples). This behavior is consistent with a decrease of PBDE usage and the current use of HNs.

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#### **References**

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