BIOMAGNIFICATION OF CLASSICAL AND EMERGING HALOGENATED FLAME RETARDANTS IN BIRD EGGS FROM DOÑANA NATURAL SPACE AND SURROUNDING AREAS

Barón, E.[1], Máñez, M.[2], Andreu, A[2], Sergio, F.[3], Hiraldo, F.[3], Eljarrat, E.[1], Barceló, D.[1,4]

[1]Water and Soil Quality Research Group, Dep. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain; [2]Natural Processes Monitoring Team, Doñana Biological Station (EBD-CSIC), c/ Américo Vespucio s/n, 41092 Seville, Spain; [3]Department of Applied Biology (EBD-CSIC), Sevilla, Spain; [4]Catalan Institute for Water Research (ICRA), H2O Building, Emili Grahit 101, 17003 Girona, Spain.

Introduction

Flame retardants (FRs) have been used for many years in order to prevent fires and, specially, halogenated compounds have shown a great efficiency in the inhibition of the combustion process. Polybrominated diphenyl ethers (PBDEs) are one of the main brominated FRs (BFRs) used and its presence has been reported in several environmental and biological matrices. Penta- and Octa-BDE formulations were banned in Europe and North America due to their bioaccumulation capacity and toxic properties. Furthermore, Deca-BDE mixture is already banned in Europe and its production in North America was stopped at the end of 2013[1].

Since the fire safety regulations needed to be reached, new compounds were developed. Some examples of emerging brominated FRs (BFRs) are hexabromobenzene (HBB), pentabromoethyl benzene (PBEB) or decabromodiphenyl ethane (DBDPE). These compounds have been found in different environmental and biological matrices in fewer amounts than the classical PBDEs. On the other hand, Dechlorane 602 (Dec-602), Dechlorane 603 (Dec-603), Dechlorane 604 (Dec-604) and Dechlorane plus (DP) were developed as an alternative for Mirex when it was banned in 1976. The first reported levels date from 2006, despite the fact that they had been used for many years. As the number of studies of these compounds increased, they proved their ubiquity as they have been found in different environmental and biological samples around the world. However, most of the studies are done close to the production sources located in the Great Lakes and China, where the concentrations are much higher than in other areas. The number of studies in areas far away from the production sources is still scarce and more information is needed.

Birds can assimilate persistent organic pollutants (POPs) through the diet and, afterwards, transfer them to the eggs, with potential consequences for offspring. Thus, eggs are considered reliable bioindicators of POPs in birds. These compounds can affect bird behaviour, the correct development of the chicks, causing malformations and reducing the shell thickness, or reproductive viability. The aim of this study was to evaluate the occurrence of the classical (PBDEs) and emerging FRs (Dechloranes, HBB, PBEB and DBDPE) in unborn eggs of 14 different species from Doñana Natural Space and surrounding areas. Differences among species and possible biomagnification processes were also evaluated using stable isotope characterization.

Materials and methods

Doñana Natural Space and surrounding areas, located in south-western Spain, is considered a sanctuary for more than 300 bird species since this area represents a strategic point where numerous birds breed, winter or stage during their migration. Bird eggs that had failed to hatch were collected during three sampling campaigns in 2010, 2011 and 2012. In total, 115 egg samples were collected corresponding to 14 different species. The number of samples per specie depended on local abundance in the three study years, since egg samples were collected opportunistically during nest checking and chick ringing operations.

1.5 gram dry weight (dw) of sample was spiked with internal standards prior to the extraction by pressurized liquid extraction (PLE) with hexane:DCM (1:1) with 2 static cycles of 10 min at 100ºC and 1500 psi. The lipid content was determined gravimetrically after the extraction and the resulting extracts were re-dissolved in hexane and treated with H_2SO_4 (conc.) to remove fat. Afterwards the organic phase was cleaned by solid phase extraction (SPE) using Al-N (5 g) cartridges. Extracts were concentrated to a final volume of 40 µL prior to the instrumental analysis[3].

PBDEs and emerging BFRs (HBB, PBEB and DBDPE) were analyzed by an Agilent 7890C gas chromatograph connected to an Agilent 5975A Network mass spectrometer, working in negative chemical ionization mode (NCI) using $NH4^+$ as reagent gas. The elution program started at a temperature of 140ºC, was held for 2 min and then ramped to 325ºC at 10ºC/min. Final temperature was held for 10 min. In order to enhance the sensitivity, selected ion monitoring (SIM) mode was applied monitoring the two most intense peaks from the NCI spectra. Ions monitored were *m/z* 79 and 81 for all PBDEs and emerging BFRs with the exception of BDE-209 and ^{13}C-BDE-209, where the two ions monitored were *m/z* 487 and 489, and *m/z* 497 and 499, respectively. The most intense peaks were used for quantification purposes, and the second ones for confirmation.

Halogenated norbornenes were analyzed using an Agilent Technologies 7890A GC system coupled to 7000A GC/MS Triple Quadrupole, working in NCI using $CH4^+$ as reagent gas. Temperature program started at 80ºC, was held for 2 min and then ramped to 300ºC in 10ºC/min. Final temperature was maintained for 10 min. Source temperature was set at 175ºC and electron energy and emission current were set at 200 and 150 eV, respectively. In order to enhance the sensitivity and selectivity, selective reaction monitoring (SRM) mode was applied. The most intense transition was used for the quantification and the second transition was used for confirmation[4].

$\delta^{13}C$ and $\delta^{15}N$ determinations

Samples were weighed to the nearest µg and placed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ determinations. Isotopic analyses were carried out at the Laboratorio de Isótopos Estables of the Estación Biológica de Doñana (LIE-EBD). All samples were combusted at 1020ºC using a continuous flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyzer coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). Stable isotope ratios are expressed in the standard δ-notation (‰) relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$). Replicate assays of laboratory standards routinely inserted within the sampling sequence, and previously calibrated with international standards, indicated analytical measurement errors of ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Results and discussion

PBDEs were detected in all the species, with total levels ranging from 1.40 (black-headed gull) to 90.7 ng/g lw (white stork). The most contaminated specie was the white stork with a mean value of 34.5 ng/g lw, followed by purple heron (mean value of 23.6 ng/g lw) and western marsh harrier (23.4 ng/g lw). The least contaminated species were the charadriiformes, anseriformes and strigiformes, with mean values between 5.03 and 6.62 ng/g lw. The three emerging BFRs included in our work, HBB, PBEB and DBDPE, were not detected in any sample. On the other hand, 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 0.77 to 260 ng/g lw. The most contaminated specie was the western marsh harrier (161 ng/g lw) but such value should be treated with caution because only one egg of this specie was collected. Excluding this unique sample, and similarly to PBDEs, the highest levels corresponded to the white stork, with a mean value of 66.1 ng/g lw, followed by black-headed gull (mean value of 63.4 ng/g lw), red kite (mean value of 45.9 ng/g lw) and slender-billed gull (mean value of 39.4 ng/g lw). Then, similar values were obtained for black kite, black-winged kite and booted eagle (mean values of 30.9, 30.7 and 29.9 ng/g lw, respectively). Slightly lower levels were found for gull-billed tern and barn owl (mean values of 23.6 and 22.6 ng/g lw). Finally, the lowest levels were for purple heron (mean value of

14.9 ng/g lw), glossy ibis (mean value of 11.8 ng/g lw), common kestrel (mean value of 8.88 ng/g lw) and gadwall (mean value of 5.93 ng/g lw). The variation in dechlorane and PBDE levels among different species was considerable, but variation was also substantial within species. Such variability is shown in Figure 1.

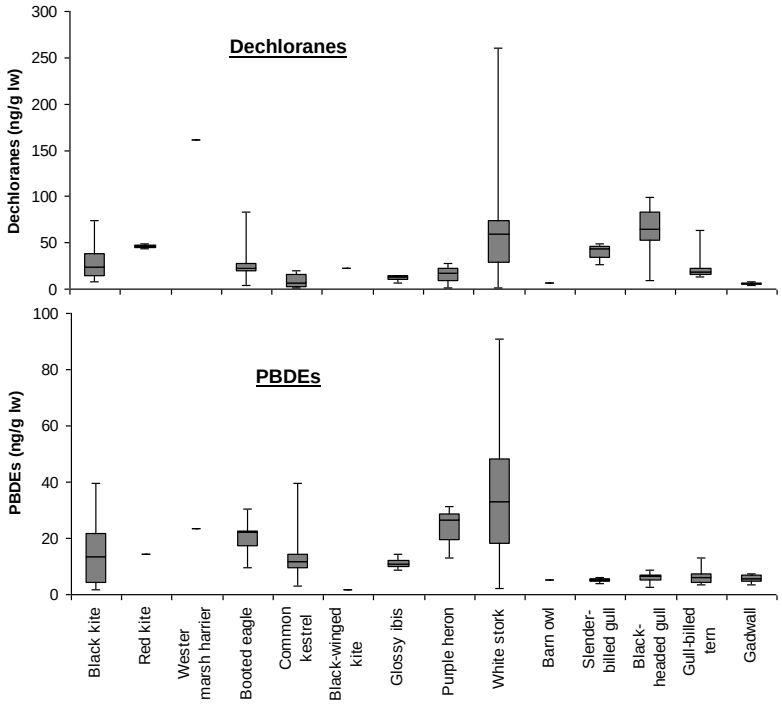

Figure 1. Box plot for PBDE and dechlorane levels for each specie.

Biomagnification study through stable isotope analysis

One powerful tool to study dietary exposure and biomagnification of contaminants in wild animal populations is the stable isotope analysis. The trophic position is defined with $\delta^{15}N$, based on the enrichment of ^{15}N throughout the food web. On the other hand, $\delta^{13}C$ is related to the food sources, providing information about the average diet of individuals over a long period. Inputs of nutrients from exogenous sources, both natural (such as nitrification or denitrification) and anthropogenic (such as human sewage and agriculture) can cause changes in baseline isotope ratios. Since water flows from Doñana National Park are susceptible of NO_3^- contamination from small urban areas in the surrounding of the park and agricultural practices allowed in the ecotone, assessing trophic levels from our $\delta^{15}N$ values must be done with caution. Based on $\delta^{13}C$ values, bird species were assigned to different habitats. However, the different species of the falconiformes presented similar values of $\delta^{13}C$ (between -21.40 and -26.32), indicating a similar behaviour regarding to the diet. On the other hand, these species presented $\delta^{15}N$ dissimilar values (between 8.95 and 14.98) enough to evaluate biomagnification processes of contaminants along trophic chain. Figure 2 shows the representation of dechlorane values in front $\delta^{15}N$. A positive correlation was found for dechloranes when the total concentration was represented against $\delta^{15}N$ (R2 = 0.67) which indicates that dechlorane levels increase as the trophic position increases, which might suggest biomagnification capacity. However, since only one sample was available for two species of the group these results should be interpreted with caution. Furthermore, it is important to note the different behaviour showed by different dechloranes. As shown in Figure 2, DP levels were not linearly correlated

with $\delta^{15}N$ (R^2 = 0.04), while a good correlation was observed for Dec-602 (R^2 = 0.75). We can conclude that Dec-602 biomagnifies along the food chain, while this behaviour is not clear for DP. In fact, previous studies showed similar results. Unfortunately, there is still a lack of information about the environmental behaviour of dechloranes in the environment. In addition, a positive correlation was found also found for PBDEs (R^2 = 0.70) which indicates that these compounds have biomagnification capacity, as has been described in previous studies. Our results show that more attention should be given to dechloranes since they show similar biomagnification behaviour as historically shown for PBDEs.

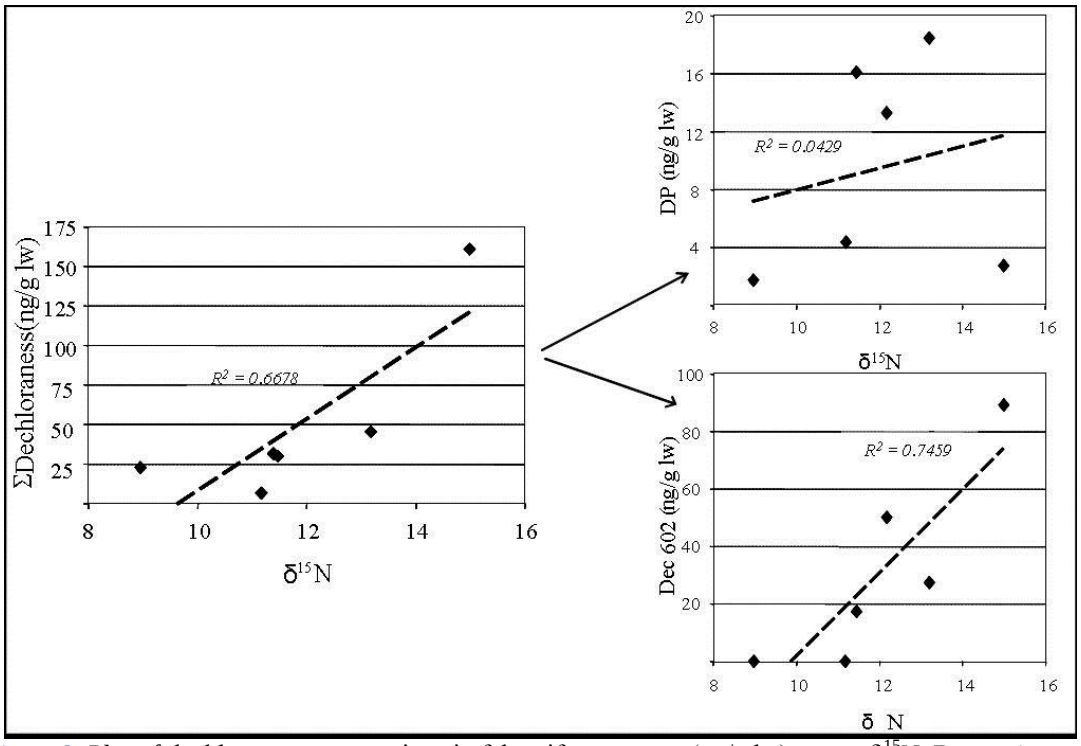

Figure 2. Plot of dechlorane concentrations in falconiformes eggs (ng/g lw) versus $\delta^{15}N$. Data points are mean values. The dotted line represents linear regression.

Acknowledgements

This work was funded by the Spanish Ministry of Environment and Rural and Marine Affairs through the project IMPAR (Ref. 106/2010), by the Spanish Ministry of Science and Innovation through the project SCARCE (Consolider Ingenio 2010 CSD2009-00065) and by the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2009-SGR-965). Sampling of raptor eggs was funded by excellence project RNM-7307 of the Junta de Andalucía and CGL2011-28103 of the Spanish Ministry of Science and Innovation. We are grateful to the Doñana Biological Station Natural Processes Monitoring Team, especially L. García, J.L. del Valle, R. Rodríguez, J.L. Arroyo, A. Martínez, A. Chico, J. Chicano, L. López and A. Tanferna for their efforts to collect bird eggs. Biotage is acknowledged for providing SPE cartridges. The authors also thank R. de Stephanis from the EBD-CSIC for the financial support with the stable isotope analysis.

References

1. M. Alaee, P. Arias, A. Sjödin, A. Bergman, Environment International 29 (2003) 683.
2. M.L. Feo, E. Barón, E. Eljarrat, D. Barceló, Analytical and Bioanalytical Chemistry 404 (2012) 2625.
3. E. Eljarrat, A. Cal, D. Barceló, Analytical and Bioanalytical Chemistry 378 (2004) 610.
4. E. Barón, E. Eljarrat, D. Barceló, Journal of Chromatography A 1248 (2012) 154.