HIGH PREVALENCE OF BDE 209 AND OTHER HIGH BROMINATED DIPHENYL ETHERS IN WHITE STORKS (*Ciconia ciconia*) FROM TWO AREAS OF SPAIN

Muñoz-Arnanz J.[1], Sáez M.[1], Pacepavicius G.[2], Alaee M.[2], Jiménez B.[1]

[1] Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC. Juan de la Cierva 3, 28006 Madrid, Spain. e-mail: bjimenez@iqog.csic.es
[2] Ecosystem Protection Research Division, Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, Canada, L7R 4A6

Abstract
Polybrominated diphenyl ethers (PBDEs) are ubiquitous pollutants for which there is still a lack of knowledge about the environmental behaviour and fate of the highly brominated congeners (octa- to deca-). In this study, the PBDE content and congener profiles in failed eggs from two colonies of white stork (*Ciconia ciconia*) in Spain were studied. The average PBDE concentrations were 1.64 $ng.g^{-1}$ for the rural colony and 9.08 $ng.g^{-1}$ for the urban colony. BDE 209 and other highly brominated congeners were detected in all samples. BDE 202, considered as an indicator of BDE 209 debromination, was detected in most samples. A predominance of the higher brominated over the lower brominated congeners in both colonies was found.

Introduction
Polybrominated diphenyl ethers (PBDEs) have been used extensively worldwide as additive flame retardants over the past several decades. They are not chemically bonded to the polymers that contain them, which facilitates their release into the environment. Despite the ban of some formulations, in many cases levels of PBDEs are still increasing in the environment[1,2], and with the ability of some BDEs to bioconcentrate and biomagnify there are concerns regarding ecosystem and human health.

Several studies on PBDE levels and congener profiles conducted on birds have shown remarkable interspecies differences[3]. To a great extent, this congener profile variability may be attributed to not only metabolic differences but also on the habitat of each species, which in turn can also be influenced by the use of commercial PBDE formulations (penta-, octa- and deca-). Diet is also an important factor influencing the differences in congener profiles between terrestrial and aquatic food webs. A common congener pattern observed in most species, especially from aquatic food webs, is dominated by BDE 47, 99, 100, 153, and 154, and at the same time, the absence of higher brominated congeners such as BDE 183 and particularly BDE 209[4]. In terrestrial food webs, the presence of BDE 183 and 209 were reported in peregrine falcons[5] in 2005. The environmental behaviour of BDE 209 is still poorly understood and it has been hypothesized that BDE 209 debrominates rapidly into lower brominated and more toxic congeners. Also, it has been postulated that it showed low oral bioavailability due to its high molecular weight and hydrophobicity, and low bioaccumulation based on its scarce presence especially in the aquatic foodweb[6]. While a number of studies detecting BDE 209 along with other highly brominated congeners in different environmental compartments are increasing, information about these compounds is still limited.

The aim of this study was to investigate a terrestrial top predator, the white stork (*Ciconia ciconia*), in terms of PBDE content and congener profile, with emphasis on the highly brominated BDE congeners. For that purpose, the archived failed eggs of two colonies of white storks from two different areas of Spain were analyzed. One of the colonies was located in Madrid, an urban and industrial area and the other colony was located in a rural area in the surroundings of Doñana National Park (DNP) considered an ecologically sensitive area and sanctuary for numerous bird species in south-western Spain.

Materials and methods

Sampling

A total of 33 failed eggs of white stork were collected. 23 of those eggs were obtained from Doñana National Park during the breeding season of 1999-2000, and 10 eggs were collected from Madrid during the breeding season of 2005. Samples were stored at -20°C until analysis. Eggs content was used for chemical analysis and the remaining eggshell was kept for further structural analysis.

Analytical procedure

Egg content was freeze-dried and quantities of approximately 2 grams were used for residue analysis. The extraction of PBDEs along with other organic pollutants involved a matrix solid phase dispersion (MSPD) procedure. Further clean-up was performed by using acid and basic silica gel multilayer columns. A final fractionation of the studied compounds and other possible interferences was achieved by using SupelcleanTM Supelco ENVI^{TM}-Carb tubes as described elsewhere[7]. Three fractions were eluted: the first fraction contained the bulk of PBDEs along with *ortho*-PCBs and DDTs, while the second and third fractions contained non *ortho* substituted PCBs and PCDD/Fs, respectively.

Analytical determination

Twelve lower brominated BDE congeners, from tri- to hepta-substituted (# 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184 and 191), were analyzed by high resolution gas chromatography low resolution mass spectrometry (HRGC/LRMS) using a 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in selected ion monitoring mode (SIM) with electron capture negative ionization (ECNI). The GC injection port was configured for 1µL pulsed hot splitless injections (4 min) at a temperature of 260ºC. Gas chromatographic separation prior to MS was achieved using a 15 m x 0.20 mm x 0.20 µm DB-5MS low bleed column (J&W Scientific, USA). The GC column was maintained at 120ºC for 4.2 min, then ramped at 30ºC/min to 200ºC, ramped again at 5ºC/min to 275ºC, ramped once again at 40ºC/min to 300ºC and maintained for 10 min, and finally ramped at 10ºC/min to 310ºC and held for 2 min. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. Methane was used as reaction gas. The temperatures of the transfer line, source and quadrupole were set at 300ºC, 150ºC and 300ºC, respectively. The identification of target compounds was based on detection, at the corresponding retention time, of the *m/z* 79 and 81 (corresponding to bromine atoms) plus two more ions corresponding to the cluster of $[M\text{-}H_xBr_y]^-$ which are specific of each congener.

Sixteen highly brominated BDE congeners, from octa- to deca-substituted (# 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208 and 209), were analyzed by high resolution gas chromatography high resolution mass spectrometry (HRGC/HRMS) using a Micromass Autospec Ultima coupled to an Agilent 6890 GC equipped with a CTC A200s autosampler. The GC injection port was configured for 1µL split/splitless injections at a temperature of 280ºC. Gas chromatographic separation prior to MS was achieved using a 15 m x 0.25 mm x 0.10 µm DB5HT column (J&W Scientific, USA). The GC column was maintained at 100ºC for 2 min, then ramped at 25ºC/min to 250ºC, ramped at 1.5ºC/min to 270ºC, ramped at 25ºC/min to 325ºC and held for 5 min. Helium was used as the carrier gas in constant pressure mode. Sample ionization was performed by electron ionization (EI) at an electron voltage ranging from 30 to 40 eV depending on the optimization parameters of the instrument. Source and transfer line temperatures were both set at 280ºC and the resolving power of the analyzer was 10,000.

Quality assurance criteria was based on the application of quality control and quality assurance measures, which included the analysis of blank samples covering the complete analytical procedure.

Results and Discussion

PBDEs were detectable in all of the samples. Out of 28 different PBDE congeners measured in this study, 17 were detected in the white storks from both colonies in at least 50% of the samples. Among those 17 congeners, 12 contained eight or more bromines, which have rarely been identified in wildlife. The relative contributions of

PBDE congeners are presented in *Figure 1*. BDE 209 has been detected previously, including in terrestrial foodwebs. Of particular interest in this study is that congener BDE 209 was detected in over 95% of the eggs and had the highest contribution to the total PBDE concentrations in both colonies.

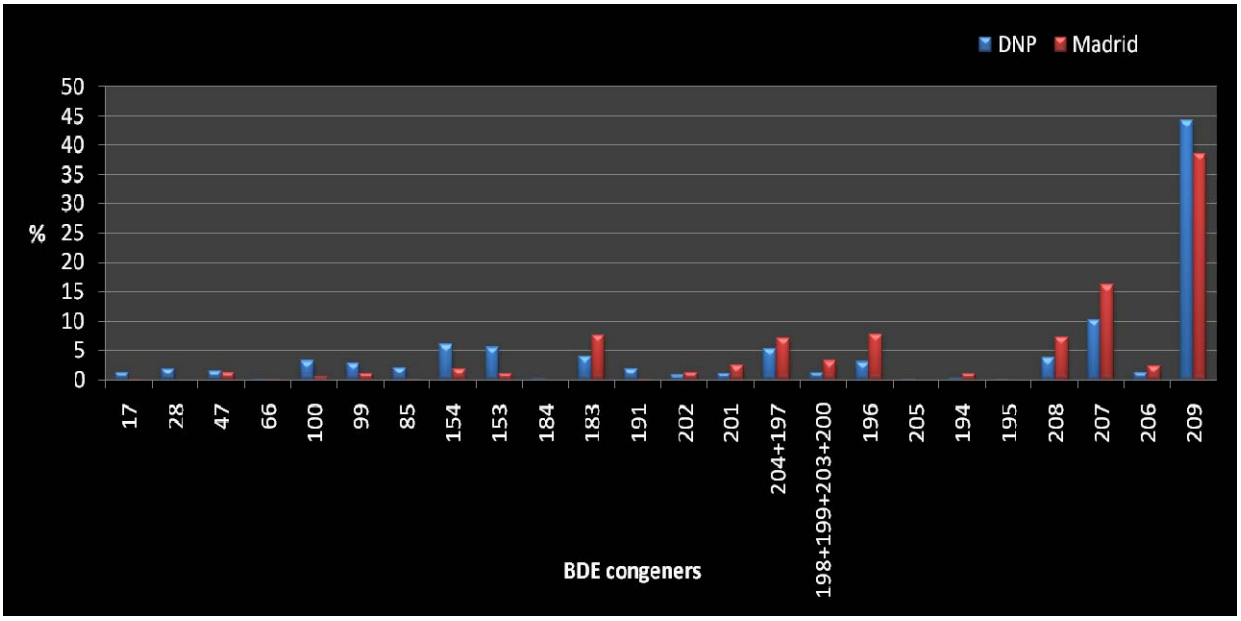

Figure 1. Relative contribution of individual congeners to the total PBDE content in white storks from Madrid (red) and DNP (blue).

The average concentration for total PBDEs in white storks from DNP was 1.64 ng.g^{-1} (median value 0.83 ng.g^{-1}) fresh weight (w.w.), and ranged between 0.21 and 9.50 ng.g^{-1}. Significantly higher levels of PBDEs (Mann-Whitney, p≤0,001) were detected in white storks from Madrid, with an average concentration of 9.08 ng.g^{-1} w.w. (median value 6.59 ng.g^{-1}) for total PBDEs, and ranged from 2.79 and 20.5 ng.g^{-1}. These results can also be affected by the fact eggs were not collected in the same year. Eggs from the Madrid colony were sampled in 2005, whereas samples from DNP colony were collected between 1999 and 2001. To date there is no study available on the levels of PBDEs in white storks; consequently, within the same species comparison is not possible. PBDE concentrations reported in this study for both colonies fall within the concentration range reported in eggs from other species of birds in the European environment, such as the great tit[8] (0.36 to 12.1 ng.g^{-1} w.w. -calculated from lipid weight-) and the peregrine falcon[9] (77 to 406 ng.g^{-1} w.w.). The reported levels are likely in part related to their relative positions in the food web and may in part be due to differences in PBDE formulations used in different areas.

Previously, a positive correlation between PCB and PBDE levels and the degree of urbanization and industrial development has been reported by Van den Oteen et al.[10] However, our previous study on PCB concentrations in the two colonies of white stork found an approximately 5-fold elevated PCB concentration in the DNP colony.[10] Therefore, it is unlikely that white storks from these colonies are exposed to similar sources for PCBs and PBDEs. The PBDE congener pattern in white storks from DNP and Madrid were dominated by higher brominated congeners. As it is shown in Figure 2, 70% and 87% of BDE congeners from DNP and Madrid respectively have eight or more bromine. These results are consistent with previous studies on terrestrial food web; and follows the hypothesis that BDE 209 bioaccumulates only to a limited extent in terrestrial birds[3]. Therefore, this study follows the pattern observed for the bioavailability of the BDE 209, where, it undergoes rapid debromination depending on the species. In this study BDE congener profile for the debromination products BDE 207 > BDE 208 > BDE 206 was observed for both colonies and they differed from the BDE

congener profiles described for the octa- and deca-BDE formulations[4]. Furthermore, our results are in agreement with data reported for both eggs from U.S peregrine falcons[11] and in a BDE 209 exposure study of the European starling[12]. Thus, it appears that part of the nona-BDEs may stem from either biodegradation or simple debromination of BDE 209. Moreover, BDE 202 was detected in all and about 83% of the eggs samples from Madrid colony and DNP colony respectively. This congener has never been detected in a commercial BDE formulation. Therefore, the presence of BDE 202 in this study indicates that this compound was formed via BDE 209 debromination in the environment. The ratio of [BDE 209] / [octa- and nona-BDEs] was 0.8 for the Madrid colony and 1.7 for the DNP colony. These differences can be attributed to different dietary habits or metabolic rates of these two colonies. Since the two colonies made up same species with different dietary habits it is more likely that the diet was the main factor contributing to these differences. This hypothesis is also backed by the congener pattern observed for lower brominated BDE congeners. Higher ratios of PBDE congeners such as 100/99, and 153/154 have been generally found to be the dominant congeners in most aquatic foodweb, in comparison to terrestrial foodweb. It is known that white storks from Madrid mainly feed at garbage dumps, whereas white storks from DNP feed on aquatic vertebrates and invertebrates, (e.g. crayfish). Therefore, these differences for lower brominated BDE congeners between the two colonies are more likely to be related to diet rather than to the different uses of technical PBDE mixtures.

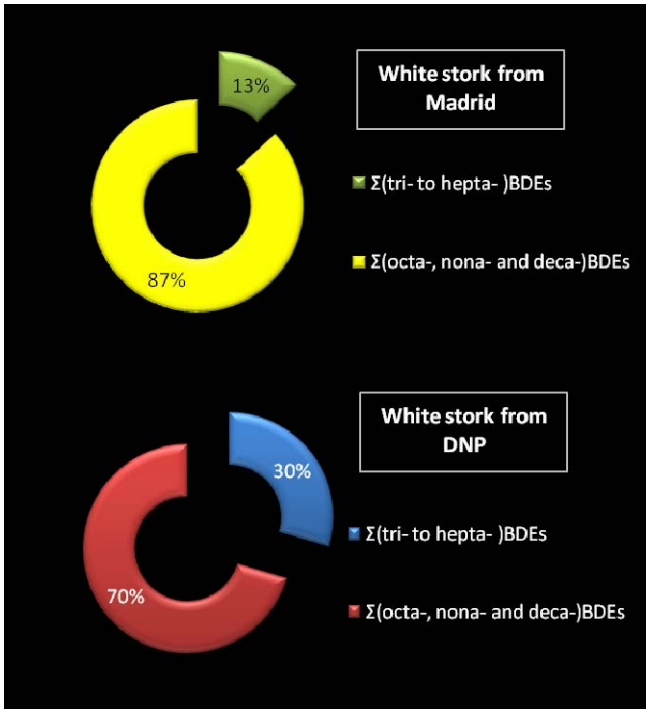

Figure 2. Contribution of low brominated (Σ(tri- to hepta-)) and high brominated (Σ(octa- to deca-)) congeners to the total PBDE content in white stork eggs from Madrid and DNP.

Acknowledgments

The authors would like to thank the Empresa de Gestión Medioambiental (EGMASA) and CSIC, (Project Intramural de Frontera Ref. 200680F0162) for their financial support. Dr. Sáez thanks the Regional Government of Madrid for her postdoctoral contract through project P-AMB-000352-0505.

References

1. de Wit C.A. *Chemosphere*. 2002;46:583-624

2. Hites R.A. 2004;38:945-956.

3. Law R.J., Allchin C.R., de Boer J., Covaci A., Herzke D., Lepom P., Morris S., Tronczynski J. and de Wit C.A. *Chemosphere*. 2006;64:187-208.

4. La Guardia M.J., Hale R.C. and Harvey E. *Environ. Sci. Technol*. 2006;40:6247-6254.

5. Jiménez B., Alaee M., Merino R. and Pacepavicius G. *Organohalogen Compounds.* 2005; 67: 518-545.

6. Stapleton H.M., Alaee M., Letcher R.J. and Baker J.E.. *Environ. Sci. Technol*. 2004;38:112-119.

7. Merino R., Bordajandi L.R., Abad E., Rivera J. and Jiménez B.. *Environ Toxicol and Chem.* 2005;24 (8): 192-197.

8. Van den Steen E., Pinxten R., Jaspers V.L.B., Covaci A., Barba E., Carere C., Cichon M., Dubiec A., Eeva T., Heeb P., Kempenaers B., Lifjeld J.T., Lubjuhn T., Mänd R., Massa B., Nilsson J-A., Norte A.C., Orell M., Podzemny P., Sanz J.J., Senar J.C., Soler J.J., Sorace A., Török J., Visser M.E., Winkel W. and Eens M. *Environ. Int.* 2009;35: 310-317.

9. Herzke D., Berger U., Kallenborn R., Nygard T. and Vetter W. *Chemosphere.* 2005;61: 441-449.

10. Muñoz-Arnanz J., Blázquez E., Ábalos M., Abad E., Rivera J., Baos R., Hiraldo F. and Jiménez B. *Organohalogen Compounds.* 2005; 70: 1562-1565.

11. Chen D., La Guardia M.J., Harvey E., Amaral M., Wohlfort K. and Hale R.C. *Environ. Sci. Technol*. 2008;42:7594-7600.

12. Van de Steen E., Covaci A., Jaspers V.L.B., Dauwe T., Voorspoels S., Eens M. and Pinxten R. *Environ. Pollu.* 2007;148: 648-653.