Environment International 37 (2011) 572–576

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Predominance of BDE-209 and other higher brominated diphenyl ethers in eggs of white stork (*Ciconia ciconia*) colonies from Spain

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ARTICLE INFO

Article history:
Received 15 March 2010
Accepted 26 November 2010
Available online 28 December 2010

Keywords:
PBDEs
BDE-209
Eggs
Birds
White stork
Doñana National Park

ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are ubiquitous pollutants for which there is still a lack of knowledge about the environmental behavior and fate of the higher brominated congeners (octa- to deca-BDEs). 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 total PBDE concentration was 1.64 ng/g (wet weight, w.w.) for the rural colony and 9.08 ng/g (w.w.) for the urban colony. Higher brominated BDEs dominated the congener profiles of both colonies. Of particular interest was the determination of BDE-209 as the dominant congener accounting for 44.1% and 38.6% of the total PBDE content in the rural and urban colonies, respectively. BDE-202, considered an indicator of BDE 209 debromination, was detected in 83% and all of the samples from rural and urban colonies, respectively. The observed congener profile in which BDE-207 > BDE-208 > BDE-206 does not correspond to any known technical PBDE mixture and is evidence for possible BDE-209 degradation.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a family of brominated flame retardants (BFRs) with 209 possible congeners of varying degrees of halogenation. These compounds have been used profusely over the past few decades to prevent fire in a wide array of consumer products including plastics, electronic circuitry, polyurethane foams, and textiles among others. The fact that these are non-matrix bound additives facilitates their release from the products that contain them. That, along with their lipophilicity and resistance to chemical degradation, has resulted in their ubiquitous distribution in the environment (Hites, 2004; Yogui and Sericano, 2007). The nature and environmental behavior of some PBDEs fulfill the criteria for being recently considered as persistent organic pollutants (POPs) under the Stockholm Convention (UNEP, 2009). Growing evidence of some BDEs' deleterious effects regarding ecosystems and human health has led to the ban of the three commercial formulations of PBDEs in the European Union. Specifically, the penta- and octa-mixtures were banned in Europe as of 2004, followed by the ban on the deca-BDE formulation in 2008 (European Court of Justice, 2008). Besides Europe, Canada banned the production of all PBDEs in 2006 (Canada Gazette, 2006) and in the United States, manufacturers voluntarily ceased production of the penta- and octa-formulations by the end of 2004 and some restrictions on the deca-BDE have already been placed in some states. Recently, the two manufacturers of deca-BDE in the United States have committed to phase out total production, importation, and sales of this formulation by the end of 2013 (US Environmental Protection Agency, 2009). With the exception of these examples in the United States, Canada, and Europe, there are no other regulations governing the production or use of the deca-BDE mixture.

Decisions to ban or phase out the deca-BDE formulation are in response to increasing information showing that BDE-209 could bioaccumulate in different organisms and degrade and metabolize into less brominated, more bioavailable, persistent, and toxic congeners (Stapleton et al., 2004; Kierkegaard et al., 2007; Van den Steen et al., 2007; Segev et al., 2009). However, even though PBDEs are one of the most studied groups of pollutants, there is still an important gap in knowledge regarding the environmental behavior and fate of BDE-209 and the higher brominated BDEs. The number of scientific papers studying these congeners is still small in comparison to the high number of studies focused on PBDEs. This may partially be due to several challenges involved in the analytical determination of these congeners (photolytic and thermal degradation), combined with a lack of commercially available analytical standards which has

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doi:10.1016/j.envint.2010.11.013

only recently been remedied (Stapleton, 2006; Kierkegaard et al., 2009).

The use of birds as bioindicators or sentinels of environmental health has been recognized for some time (Furness, 1993). Birds that are near or at the top of the food chain are susceptible to bioaccumulation and biomagnification. Consequently, these species may be more sensitive to the effects of contaminant exposure and bioaccumulation. The use of infertile eggs has been extensively reported and regarded as a useful non-destructive tool for the study of contaminants in bird populations (Merino et al., 2005; Jiménez et al., 2007). Regarding PBDE content and congener profiles, several studies conducted on birds have revealed remarkable interspecies variability (Law et al., 2006). These differences are attributed not only to distinct metabolic rates but also to diet and habitat since the availability and use of PBDEs in different ecosystems and environments influences the exposure of birds to PBDEs.

For instance, most avian species having an aquatic-based diet show a common congener profile dominated by BDE-47, -99, -100, -153, and -154. Moreover, if detected, higher brominated BDE congeners such as BDE-183 and particularly BDE-209 are found at very low concentrations. This has been partially explained by the low bioavailability of BDE-209 due to its high molecular weight and hydrophobicity and its tendency to bind to soil and sediment. In terrestrial food webs, the presence of BDE-183 and -209 was first reported in 2004 by Lindberg et al. in top predators such as peregrine falcons. Since then, several studies have detected levels of BDE-209 and some nona-, octa- and hepta-BDEs in falcons and other birds of prey from Norway (Herzke et al., 2005), Switzerland (Naert et al., 2007), Sweden (Johansson et al., 2009), China (Chen et al., 2007; Gao et al., 2009), and the United States (Chen et al., 2008; Holden et al., 2009; Chen et al., 2010). With the exception of some birds of prey from China and the United States, both the reported concentrations of higher brominated BDEs, including BDE-209, and their contribution to the total PBDE content have been low.

The objective of this study was to investigate the PBDE content and congener profile in the white stork as a top predator with different feeding habits depending on the foraging area. Special attention was paid to the higher brominated BDE congeners. For that purpose, white stork eggs were obtained from two colonies with marked differences in terms of their habitats. One of the colonies (Madrid colony) was located in an urban/industrial area, near the city of Madrid, where nests were built in anthropogenic habitats where the presence of rubbish dumps could provide the storks with a constant food source (Martínez, 1995). The other colony inhabited Doñana National Park (DNP) and its surroundings (DNP colony) in southwestern Spain, which is considered an ecologically sensitive rural area and sanctuary for numerous bird species. In this colony, white storks were observed to breed in open nests at the top of wild olive trees located in a natural area far from urban or industrial influences. Therefore, the DNP storks consume a natural diet involving terrestrial and aquatic species such as crayfish, terrestrial insects, earthworms, and amphibians among others.

2. Materials and methods

2.1. Sample collection

A total of 33 addled eggs of white stork were collected. Twenty-three were obtained from Doñana National Park during the breeding seasons of 1999–2000, and ten eggs were collected from Madrid during the breeding season of 2005 (Fig. 1). Nests were carefully monitored during the breeding period and all of them were sampled at the same time intervals, so it was assumed that all eggs had the same water loss. Samples were stored at −80 °C until analysis. Before residue analysis, eggs were examined, and none of them was embryonated. Egg content was used for chemical analysis and the remaining eggshell was kept for further structural analysis.

2.2. Analytical procedure

The whole egg content was lyophilized and quantities of approximately 2 g were used for residue analysis according to the analytical procedure described in detail elsewhere (Merino et al., 2005). Briefly, samples were spiked with ^{13}C-labeled surrogate standards and the extraction was based on a matrix solid phase dispersion (MSPD) procedure. Further cleanup was performed by using acidic and basic silica gel multilayer columns. A final fractionation of the studied compounds and other possible interferences was carried out by using Supelclean™ Supelco ENVI™-Carb tubes. Three fractions were eluted: the first fraction contained the bulk of PBDEs along with ortho-PCBs and DDTs, whereas the second and third fractions contained non-ortho substituted PCBs and PCDD/Fs, respectively. The first fraction containing PBDE congeners was used in the present work. Non-ortho-PCBs and PCDD/Fs obtained in the second and third fractions constituted

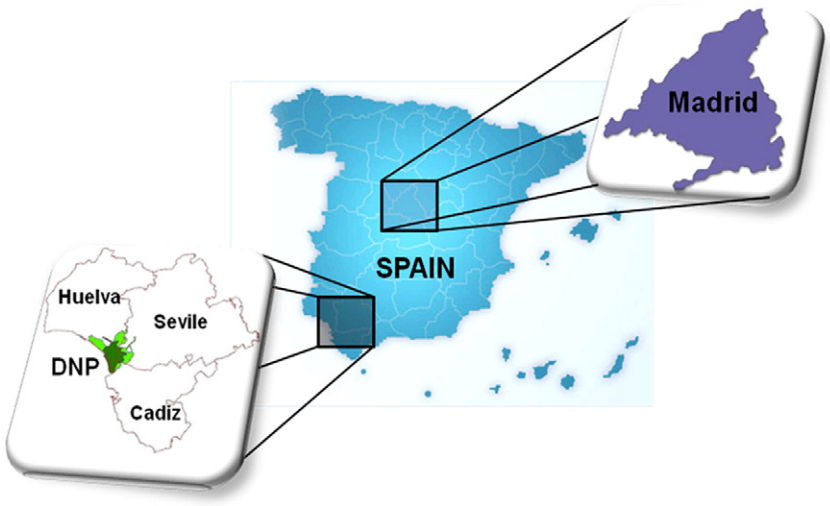

Fig. 1. Geographic distribution of the white stork colonies sampled. DNP stands for Doñana National Park.

part of a separated work (Muñoz-Arnanz et al., 2008). Lipid content of each sample was calculated gravimetrically (additional details provided in Supplementary Information).

Prior to instrumental analysis, $^{13}C_{12}$-BDE 138 or $^{13}C_{12}$-BDE 139 were added to all extracts as injection standards to correct for instrument variability. Twelve tri- to hepta-substituted PBDE congeners (BDE-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 6890 N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA) operated in selected ion monitoring mode (SIM) with electron capture negative ionization (ECNI). The GC injection port was configured for 1 μL pulsed hot splitless injections (5 psi during 4 min) at a temperature of 260 °C. Gas chromatographic separation prior to MS was achieved using a 15 m × 0.20 mm × 0.20 μm DB-5MS low bleed column (J&W Scientific, USA). The GC column was maintained at 120 °C for 4.2 min, then ramped at 30 °C/min to 200 °C, ramped again at 5 °C/min to 275 °C, ramped once again at 40 °C/min to 300 °C and maintained for 10 min, and finally ramped at 10 °C/min to 310 °C 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 300 °C, 150 °C, and 150 °C, respectively. The identification of target compounds was based on detection, at the corresponding retention time, of m/z 79 and 81 (corresponding to bromine atoms) plus 2 more ions corresponding to the cluster of $[M\text{-}H_xBr_y]^-$ which were specific to each congener.

Sixteen higher brominated PBDE congeners, from octa- to deca-substituted (BDE-194, -195, -196, -197 + 204, -198 + 199 + 200 + 203, -201, -202, -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 1 μL split/splitless injections at a temperature of 280 °C. Gas chromatographic separation prior to MS was achieved using a 15 m × 0.25 mm × 0.10 μm DB-5HT column (J&W Scientific, USA). The GC column was maintained at 100 °C for 2 min, then ramped at 25 °C/min to 250 °C, ramped at 1.5 °C/min to 270 °C, ramped at 25 °C/min to 325 °C and held for 5 min. Helium was used as the carrier gas in constant pressure mode. Analyte 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 280 °C and the resolving power of the analyzer was 10,000. Quality assurance/control criteria are provided in the Supplementary Information.

2.3. Data analysis

All concentrations are given in wet weight (w.w.). For comparative purposes, the mean content of lipids in the analyzed eggs was 7.32 ± 0.96 % in the case of DNP and 7.18 ± 0.73 % in the case of Madrid. Samples with concentrations below the detection limits were assigned a value of zero. Statistical analyses were carried out with SigmaPlot for Windows version 11.0 (Systat Software Inc., CA, USA). Data were not normally distributed (Shapiro–Wilk test, $p<0.05$). The data were $\log_{10}$-transformed in order to meet normality. The level of significance was set at $\alpha = 0.05$.

3. Results and discussion

3.1. Congener patterns and levels

PBDEs were found in all the samples analyzed (Table 1). Of the 28 different PBDE congeners measured in this study, 17 were in at least 50% of the white stork eggs. The following fifteen congeners were found in both colonies: BDE-154,-183,-196, -197, -198, -199, -200, -201, -202, -203, -204, -206, -207, -208, and 209. Interestingly, 12 of these 15 congeners contained 8 or more bromine atoms which had only been scarcely reported in wildlife to date. It is also worth noting that BDE-47, -99,-100, and -153, often dominant in biological samples, were not detected in some samples (Table 1). The study of the relative contributions of PBDEs revealed a marked presence of deca-, nona-, and octa-BDEs in comparison to the rest of congeners (Fig. 2). Specifically, 70% and 87% of BDE congeners from DNP and the Madrid colony, respectively, had 8 or more bromine atoms. Of special relevance is the contribution of BDE-209 which has been previously reported in terrestrial food webs. In this study, however, not only was it detected in over 95% of the eggs analyzed but it also accounted for the highest contribution to the total PBDE content in both colonies.

The average concentration for total PBDEs in white storks from DNP was 1.64 ng/g w.w. (median value, 0.832 ng/g), ranging between 0.214 and 9.50 ng/g. The levels found for

Table 1
Arithmetic mean, median, range and detection frequencies (% > LOD) of PBDE concentrations (ng/g w.w.) in white stork eggs from the colonies of Madrid and DNP. ND: Not Detected.

	DNP ($n=23$)				Madrid ($n=10$)			
Congeners	Mean	Median	Range	Percentage>LOD	Mean	Median	Range	Percentage>LOD
BDE-17	0.019	0	ND–0.218	13.6	0.0003	0	ND–0.003	10
BDE-28	0.029	0.010	ND–0.183	59.1	ND	ND	ND	0
BDE-47	0.023	0	ND–0.172	36.4	0.107	0.111	ND–0.150	90
BDE-66	0.0001	0	ND–0.003	4.55	0.0009	0	ND–0.008	10
BDE-85	0.032	0	ND–0.674	22.7	0.008	0	ND–0.069	20
BDE-99	0.046	0	ND–0.339	50	0.089	0	ND–0.311	30
BDE-100	0.052	0	ND–0.460	27.3	0.034	0	ND–0.114	30
BDE-153	0.090	0	ND–0.522	40.9	0.086	0	ND–0.389	30
BDE-154	0.097	0.037	ND–0.744	95.5	0.161	0.156	0.009–0.259	100
BDE-183	0.064	0.041	0.003–0.289	100	0.690	0.629	0.309–1.24	100
BDE-184	0.003	0	ND–0.023	18.2	ND	ND	ND	0
BDE-191	0.030	0	ND–0.330	31.8	0.003	0.007	ND–0.020	30
BDE-194	0.004	0	ND–0.070	17.4	0.086	0.081	ND–0.146	88.9
BDE-195	0.0003	0	ND–0.007	4.35	ND	ND	ND	0
BDE-196	0.048	0.031	ND–0.187	95.7	0.703	0.553	0.166–1.60	100
BDE-197 + 204	0.086	0.065	ND–0.287	95.7	0.638	0.521	ND–1.27	88.9
BDE-198 + 199 + 200 + 203	0.017	0.014	ND–0.074	82.6	0.286	0.153	0.064–0.840	100
BDE-201	0.016	0.011	ND–0.079	95.7	0.224	0.158	ND–0.505	88.9
BDE-202	0.013	0.008	ND–0.086	82.6	0.105	0.087	0.023–0.292	100
BDE-205	0.0002	0	ND–0.005	8.70	ND	ND	ND	0
BDE-206	0.019	0.010	ND–0.162	65.2	0.218	0.126	0.036–0.952	100
BDE-207	0.167	0.072	ND–1.24	91.3	1.47	1.20	0.435–3.85	100
BDE-208	0.060	0.024	ND–0.465	91.3	0.657	0.498	0.157–1.91	100
BDE-209	0.724	0.327	ND–6.74	95.7	3.50	2.71	0.603–8.46	100
ΣPBDEs	1.64	0.832	0.214–9.50		9.08	6.59	2.79–20.5	

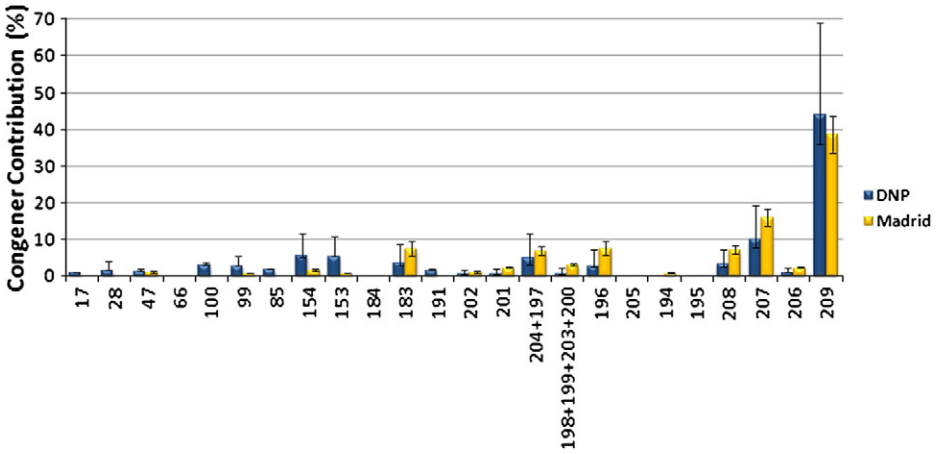

Fig. 2. PBDE congener profiles of both colonies of white stork. Error bars represent 25 and 75 percentiles.

the Madrid colony were significantly higher ($p<0.001$), with an average concentration of 9.08 ng/g w.w. (median value, 6.59 ng/g) for total PBDEs and ranging from 2.79 to 20.5 ng/g. Since a positive correlation between PBDE levels and the degree of urbanization and industrial development has been previously described (Chen et al., 2008), these results were a priori expected given the differences in the levels of anthropogenic influences between the two sites (i.e. rural vs. urban/industrial). To the best of our knowledge, there are no previous studies reporting PBDE concentrations in white storks, making it impossible to evaluate temporal trends within this species. In addition, it is important to note that these eggs were not collected in the same year—the eggs from DNP were collected during the breeding seasons of 1999–2001 and those from the Madrid colony were sampled in 2005. However, concentrations of PBDEs measured in the stork eggs from both colonies are within the range reported for eggs of other avian species of the European continent having lower and higher trophic positions than the white storks. Concentrations in the white stork eggs were similar to the concentrations reported in the eggs of the great tit (0.36–12.1 ng/g w.w.), which is a species at a lower trophic position and that consumes a strictly terrestrial diet of invertebrates and plant parts (Van den Steen et al., 2009). Compared to PBDE concentrations in the peregrine falcon eggs (77–406 ng/g w.w.), a species that has one of the highest trophic positions since it consumes other avian species, PBDE levels in the eggs of the storks were lower (Herzke et al., 2005).

In light of these values, it seems likely that PBDE levels for white storks are partially related to their relative position in the food web. Nevertheless, the possible influence of different PBDE mixture uses in different areas cannot be disregarded.

3.2. Differences among colonies

There were distinct differences between the two colonies in relation to the contribution of each homolog group to the total ΣPBDEs (Fig. 3). Contrasting with the higher BDE-209 burden in the Madrid colony, the ratio [BDE-209]/[octa- and nona-BDEs] was 0.8 for the Madrid colony and 1.7 for the DNP colony. These differences may be attributed to different dietary habits or metabolic rates of these two colonies. Since both colonies are made up of the same species with different dietary habits, it is more likely that diet was the main factor contributing to these differences. This hypothesis is also confirmed by the congener pattern observed for lower brominated BDE congeners. A higher abundance of congeners such as BDE-47, -99, -100, -153, and -154 have been generally found in most aquatic food webs in comparison to terrestrial food webs. This is the particular case for most of these lower BDE congeners for DNP storks in comparison to the Madrid specimens (Figs. 2 and 3). It is known that white storks from Madrid mainly feed at garbage dumps, whereas white storks from DNP partially 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 use of different technical PBDE mixtures.

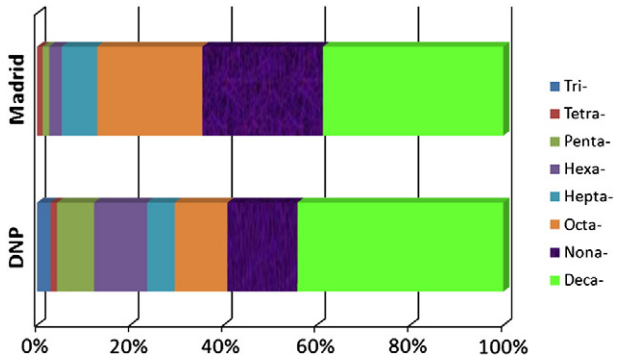

Fig. 3. Relative contribution of each PBDE homolog group to ΣPBDEs in each colony.

3.3. BDE-209. Bioaccumulation and debromination

BDE-209 has been previously reported as the dominant congener in some terrestrial species such as red foxes (Voorspoels et al., 2006) and grizzly bears (Christensen et al., 2005). To date, BDE-209 has been only detected as the dominant congener in some terrestrial birds of prey in China (Chen et al., 2007; Gao et al., 2009) and the United States (Park et al., 2009). In this study, the BDE-209 contribution to the total PBDE content was as high at 38.6% and 44.1% for the Madrid and DNP white storks, respectively. This is a remarkable finding that supports growing evidence that BDE-209 is bioavailable and has a tendency to bioaccumulate in some terrestrial species. Our results also agree with those reported by Chen et al. (2007) who found that among those species for which BDE-209 accounted for the highest contribution, the most contaminated birds showed the lowest BDE-209 relative burdens. The relative contribution of BDE-209 in DNP white storks is statistically higher ($p<0.001$) than that of Madrid white storks, whereas the ΣPBDEs is statistically greater for the latter birds. This finding, however, contrasts with data reported by Park et al. (2009), who found not only greater PBDE contents in urban peregrine falcons but also greater BDE-209 contributions. In that study, the authors support their findings postulating a direct uptake of BDE-209 in urban food webs based on fewer biological transfers. According to this hypothesis, fewer biological transfers would indicate a less preferential uptake of lower brominated BDEs in comparison to BDE-209. While this may be correct, in the case of the white stork, a higher relative content of BDE-209 corresponds to a lower relative content of nona-BDEs and vice versa. Again, this distinction between the two white stork populations is likely to be associated with uptake differences rather than dissimilar metabolic rates.

The detection of some of the higher BDE congeners may serve to further support the existing evidence for BDE-209 biodegradation or/and environmental debromination. Thus, the congener profile found in the white storks for the debromination products BDE-207>BDE-208>BDE-206 differed from the congener profiles described for the technical octa-BDE (BDE-207>BDE-206>BDE-208) and deca-BDE formulation (BDE-206>BDE-207>BDE-208) (La Guardia et al., 2006). Moreover, it also differed from the common profile found in abiotic matrices where BDE-206 is detected as the dominant nona-BDE congener (Holden et al., 2009). Our results are in agreement with data reported for eggs from US peregrine falcons (Chen et al., 2008) and European starlings in a BDE-209 exposure study (Van den Steen et al., 2007), suggesting that part of these nona-BDE congeners may stem from either biodegradation or simple debromination of BDE-209. The congener BDE-202 was detected in all and about 83% of the egg samples from the Madrid and DNP colonies, respectively. The presence of this congener has never been reported in any commercial PBDE mixture (Stapleton et al., 2006). Ruling out this technical origin, the detection of BDE-202 in the white stork eggs studied may result from two different sources as follows: (i) debromination of BDE-209 in the environment and subsequent uptake and bioaccumulation and/or (ii) as a metabolic product of higher BDE congeners. BDE-202 has been found previously in other studies on biota and postulated as a BDE-209 debromination product (Christensen et al., 2005).

3.4. Toxicological considerations

It is known that, in general, for birds and other oviparous species, the transfer from the mother to eggs of those contaminants with low Kow is favored over those compounds with a higher Kow value (Wu et al., 2009). Other parameters such as the molecular geometry and size, the degree of halogenation, or the rate of metabolism greatly influence the transfer as well. As a result, each group of organohalogen compounds may exhibit a different behavior in the maternal transfer. Specifically for PBDEs, it has been described how the transfer of the higher brominated BDE congeners is hindered in glaucous gulls (Verreault et al., 2006). Assuming a similar outcome in the maternal transfer for white storks, the relative weight of octa-, nona-, and deca-BDEs into the total PBDE content of adult white storks could be greater than what has been detected in eggs.

Several studies exist that have found a negative correlation between ΣPBDEs and reproductive success in different bird species (Fernie et al., 2009). The concentrations found for both colonies of white stork in this study are far below the suggested threshold of 1000 ng/g associated with reduced reproductive performance in ospreys (Henny et al., 2009). Nevertheless, the different interspecies sensitivity towards contaminants cannot be ignored. This, together with the increasing evidence of bioaccumulation and debromination of the higher brominated BDE congeners, should be an element taken into account to further support worldwide restriction/regulation policies on the deca-BDE technical formulation.

4. Conclusions

The present study emphasized BDE burdens dominated by the higher BDE congeners in two colonies of white stork from different areas of Spain. The contribution of BDE-209 was remarkably high in birds from these two colonies, a finding scarcely reported to date. The abundance of other higher BDEs, such as nona-BDEs, was markedly different in both colonies, suggesting distinctly different uptake of the congeners that was likely heavily influenced by dietary differences between the two colonies. The congener profile BDE-207>BDE-208>BDE-206, along with the presence of BDE-202, found in the white stork eggs analyzed in this study further supports the hypothesis that BDE-209 undergoes biodegradation or/and environmental debromination to lower BDE congeners. Results from this study contribute to increase the environmental information on DecaBDE and therefore could help to support decisions to ban or phase out the deca-BDE formulation.

Acknowledgments

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2010.11.013.

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