Emerging Brominated Flame Retardants in White Storks (*Ciconia ciconia*) Colonies from Spain

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Introduction

Injury, death, property destruction and economic loss are all consequences of fires. For example, in the US, in 2007 over 1.5 million fires were reported, which resulted in 17675 injuries, 3430 deaths, and direct losses of over $14 billion (National Fire Protection Association, 2009). Flame-retardants are added to consumer products to decrease their inherent flammability hence providing a better opportunity for survival. Over 175 compounds have been designated as flame retardants (Alaee et al. 2003) of which brominated flame retardants (BFRs) are of particular interest. Currently more than 80 compounds have been registered in commerce as BFRs. Over the past three decades, some BFRs have been detected in environmental matrices and human tissue which can be attributed to the anthropogenic uses of these compounds. This has resulted in bans of polybrominated biphenyls (PBBs) and diphenyl ethers (PBDEs) in several jurisdictions and voluntary withdrawals in other jurisdictions. Consequently, there has been a move towards increasing the use of other BFRs thus making it essential to look for these compounds in environmental matrices.

Species that are at high levels of the food web have often been used as bioindicators to evaluate the presence of persistent contaminants in ecosystems. Trophic level plays an important role when a species is selected as "environmental indicator". Consequently, top predators are more exposed to bioaccumulative contaminants resulting in higher concentrations and more impact. Among wildlife, birds have been suggested as useful organisms for monitoring pollutant levels. Very often, such studies have been carried out with raptors (Jiménez et al. 2007, Gómara et al. 2008). However, other species such as white stork (*Ciconia ciconia*) have also shown their usefulness as sentinels of environmental health (Sáez et al. 2008, Muñoz-Arnanz et al. 2008). White Stork is a colonial species that prey predominantly on wildlife; however, recently rubbish dumps have become an important source of nutrients which could greatly influence their contaminant burden. The use of infertile eggs has been extensively considered as a useful non-destructive tool for the study of contaminants in bird populations (Jiménez et al. 2007, Gómara et al. 2008). Recently we demonstrated the occurrence, bioaccumulation and debromination of BDE 209 in infertile white stork eggs from two colonies in Spain (Muñoz-Arnanz et al. 2010).

The objective of this study was to investigate the occurrence of emerging BFRs in white stork as a top predator. For that purpose, addled eggs were obtained from two colonies with marked differences in terms of their foraging areas. One of the colonies (Madrid colony) was located in an urban/industrial area near the city of Madrid where the presence of rubbish dumps could provide a constant food source. The other colony inhabited Doñana National Park (DNP) and its surroundings (DNP colony) in south-western Spain which is considered an ecologically sensitive area and sanctuary for numerous bird species. In this colony, white storks breed in a natural area far from urban or industrial influences with a diet based on natural resources.

Materials and Methods

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. Samples were stored at -20°C until analysis. Egg content was lyophilized and quantities of approximately 2 grams were used for residue analysis following the procedure described elsewhere (Merino et al. 2005).

Fifteen BFRs (Table 1) 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.

Table 1. List of the 15 emerging BFRs and their acronyms analyzed in this study.

BFR	Compound
ATE	allyl 2,4,6-tribromophenyl ether
bTBECH	β-tetrabromoethylcyclohexane
pTBX	2,3,5,6-tetrabromo-p-xylene
BATE	2-bromoallyl 2,4,6-tribromophenyl ether
PBT	pentabromotoluene
PBEB	pentabromoethylbenzene
DPTE	2,3-bromopropyl 2,4,6-tribromophenyl ether
HBB	hexabromobenzene
EHTBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate
BB-153	2,2',4,4',5,5'-hexabromobiphenyl
BTBPE	1,2-bis(2,4,6-tribromophenoxy) ethane
BEHTBP	bis(2-ethyl-1-hexyl) tetrabromophthalate
OBIND	octabromotrimethylphenylindane
DBDPE	decabromodiphenylethane
HCDBCO	hexachlorocyclopentenyl-dibromocyclooctane

Results and Discussion

Of the 15 studied BFRs, only BB-153, PTB and PBEB were detected in both colonies. Moreover, important differences regarding detection frequencies were found generally both in terms of studied compounds and colonies. In the Madrid colony, BB-153, PBT and PBEB were detected in 100, 90 and 20% of the egg samples, whereas the frequency of detection for the same species in the DNP colony was 100, 70 and 13%, respectively. The average concentrations, median and range are shown in Table 2 along with BDE-209 for comparison purposes.

PBT and PBEB concentrations found in Madrid were higher than those found in the DNP colony (Figure 1) while BB-153 was higher in DNP. Yet, these differences were not statistically significant (Mann-Whitney, $p<0.05$). In the case of PBEB, this is likely due to the low number of samples in which it was detected in both colonies. However, for BB-153 and PBT, which were detected at much higher frequencies, it is worth noting that their levels do not seem to correlate with the foraging area, i.e. rural vs. urban/industrial. This is contrary to what has been previously

found for PBDEs; where the average concentration of PBDEs in the urban/industrial colony (Madrid) was about 5-fold larger than that detected in the rural colony (DNP) (Muñoz-Arnanz et al. 2010).

Table 2. Mean, median and range of BFR concentration in white stork eggs from the DNP and Madrid colonies.*BDE-209 content obtained from (Muñoz-Arnanz et al. 2010).

		Mean	Median	Range
			pg/g w.w.	
DNP (n=23)	BB-153	44.2	38.3	3.61 – 149
	PBT	0.98	1.28	0.01 – 5.10
	PBEB	0.60	0.83	0.01 – 1.18
	BDE-209*	720	330	ND – 6740
Madrid (n=10)	BB-153	32.0	21.8	14.8 – 82.2
	PBT	1.58	0.71	0.61 – 2.95
	PBEB	5.47	6.11	1.14 – 9.79
	BDE-209*	3500	2710	600 – 8460

Interestingly HBB was detected in about 22% of the samples from DNP colony, with an average concentration of 2.39 pg/g w.w. (median value 3.08 pg/g), ranging between 0.27 and 7.79 pg/g. Additionally, BATE and OBIND were detected in one egg sample each, with values of 4.24 and 10.1 pg/g, respectively.

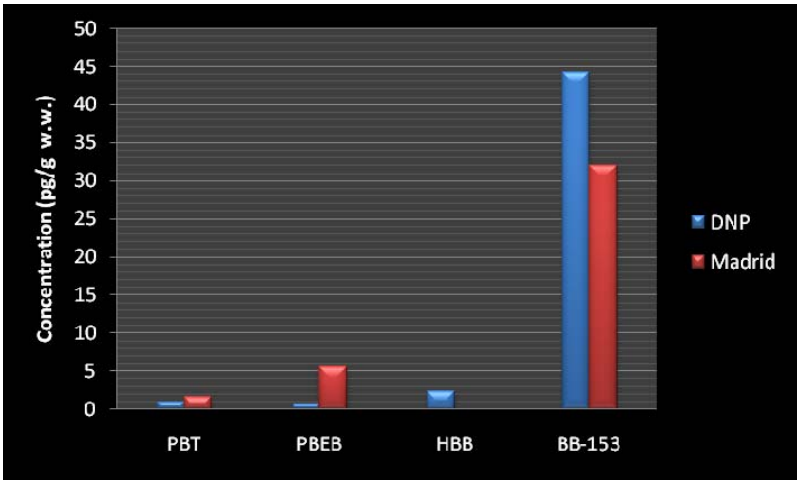

Figure 1. Concentration of the most abundant BFRs detected in white stork eggs from the DNP and Madrid colonies.

In terms of absolute values, emerging BFR levels in both colonies were significantly lower (up to three orders of magnitude) than what has been reported in eggs of herring gulls (*Larus argentatus*) from the Great Lakes (Gauthier et al.2007), and in glaucous gulls (*Larus hyperboreus*) even from pristine areas such as the Norwegian Arctic (Verreault et al.2007) as is shown in Table 3. Likewise, BFR levels measured in this study were up to four orders of magnitude lower than those of BDE-209 measured in the same samples in a previous study (Table 2).

Table 3. Concentration of selected emerging BFRs in herring gulls eggs.

		Range pg/g w.w.
Herring Gulls from Laurentian Great Lakes	HBB	240 – 530
	PBT	4 – 20
	PBEB	30 – 1400
Glaucous Gulls from Norwegian Arctic	HBB	420 – 2640
	PBT	ND – 120
	PBEB	30 – 230

Regardless of the low concentrations measured, the presence of emerging BFRs in this study in white stork eggs from significantly different colonies further supports evidence for the widespread environmental distribution, bioaccumulation and maternal transfer potential of some of these new and unregulated compounds.

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