Halogenated flame retardants in birds from central Spain: PBDE levels still very high

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Introduction

Birds can assimilate POPs through dietary exposure and trophic transfer. In addition, there is an associated phenomenon of maternal transfer of POPs from female to eggs and subsequently in embryos and hatchlings. Thus, eggs are considered reliable bioindicators of POPs. In birds, these compounds cause various behavioural, neurological and physiological abnormalities that affect the reproduction and health of species and ultimately affect the population dynamics.

The aim of this study was to evaluate the occurrence of classical and emerging halogenated FRs (HFRs) in different species of birds collected from Castrejón reservoir, Tajo river (Toledo, central Spain). It is relevant to evaluate if legal restrictions over PBDEs and the consequent increase in the use of emerging HFRs have been reflected on bird concentrations. Finally, it is important to compare current levels of contamination by PBDEs with lowest observed effect concentration (LOEC) values reported in the literature, in order to determine if current PBDE concentrations are still associated with possible ecological hazards.

Materials and methods

Several bird eggs that had failed to hatch were collected during 2010 and 2017. Egg samples were collected opportunistically during nest checking and chick ringing operations, so that the number of samples per species depended on local abundance in each sampling year. In total, 33 egg samples were collected corresponding to 4 different species. Three species corresponded to Pelecaniformes Order: grey heron (*Ardea cinerea*), purple heron (*Ardea purpurea*) and black-crowned night heron (*Nycticorax nycticorax*). Moreover, one sample of western marsh harrier (*Circus aeruginosus*), corresponding to the Accipitriformes Order, was also available. All eggs were frozen and sent to the laboratory in individual and protected containers.

Egg samples were measured, broken, weighted, homogenized and freeze dried. 1.5 gram dry weight (dw) was spiked with labeled internal standards. Pressurized liquid extraction (PLE) with a mixture of hexane: DCM (1:1), 2 static cycles of 10 min. at 100ºC and 1500 psi was used. Then, the lipid content was determined gravimetrically and the resulting extracts were re-dissolved in hexane and treated with H_2SO_4 (conc.) to remove fat. After, the organic phase was cleaned by solid phase extraction (SPE) using Al-N (5 g) cartridges. Extracts were evaporated to incipient dryness and reconstituted to a final volume of 40 µL. PBDEs and dechloranes (DECs) were analyzed by a GC-MS Triple Quadrupole. Chromatographic separation was carried out with a DB-5ms column (15m×0.25mm×0.1 µm). For PBDEs, electron ionization (EI) was applied, whereas DECs were analyzed using negative chemical ionization (NCI). Due to low sensitivity to decabrominated analytes using GC-EI-MS-MS, BDE-209 was determined with GC-NCI-MS. Recoveries between 59-99% were obtained, with reproducibility between 1.3-22%. Method limits of detection (mLODs) and method limits of quantification (mLOQ) ranged from 0.002 to 3.19 and from 0.008 to 10.6 ng/g lipid weight (lw), respectively.

Results and discussion

PBDEs were detected in all the species and all the bird egg samples. Total levels ranged from 22.2 to 5167 ng/g lw (Table 1). The most contaminated species was the grey heron with a mean value of 1912 ng/g lw, followed by purple heron (mean value of 692 ng/g lw) and western marsh harrier (645 ng/g lw), and finally, black-crowned night heron (243 ng/g lw). On the other hand, DECs were also detected in all the species, but in the 79% of the analyzed samples. Total levels ranged from nd to 132 ng/g lw, being lower than the corresponding PBDE levels. And similar to what was observed for PBDEs, the species with the highest DEC levels was grey heron (mean value of 41.4 ng/g lw) followed by purple heron (32.8 ng/g lw). The variation in PBDE and DEC levels among the different species was considerable, but we must also highlight the variability within the different egg samples of the same species. In birds, factor such as age, body condition and habitat may affect the contaminants accumulated by the female, which are transferred to the egg. The highest levels found in grey heron samples could indicate that the greatest contamination can be attributed to the study area, that is, Toledo (Spain). The other two species, purple heron and black-crowned night heron, being migratory, will be receiving a lower impact during their migratory period in sub-Saharian Africa. Moreover, the lowest levels of black-crowned night heron samples could be related to their feeding habits since they feed on smaller animals, placing itself at a somewhat lower trophic level than purple heron.

Table 1. Concentration values (expressed in ng/g lw) of PBDEs and DECs in bird egg samples.

		BDE-28	BDE-47	BDE-100	BDE-99	BDE-154	BDE-153	BDE-183	BDE-209	**ΣPBDEs**	Dec 602	Dec 603	Dec 604	*syn* -DP	*anti* -DP	**ΣDECs**
Grey heron (*Ardea cinerea*)																
2011	B-AC-1	1,71	449	251	363	109	107	39,7	6,62	**1327**	41,7	1,29	nd	0,97	2,29	**46,2**
2011	B-AC-2	16,1	1699	577	1434	141	327	93,2	2,68	**4292**	69,6	3,08	nd	1,07	2,16	**76,0**
2012	B-AC-3	7,29	844	228	586	99,2	146	67,1	16,4	**1994**	99,4	18,2	nd	5,00	9,52	**132**
2015	B-AC-4	11,6	357	404	562	96,3	269	52,5	1,19	**1754**	0,13	2,12	nd	2,18	5,62	**10,0**
2015	B-AC-5	31,4	1551	1125	1544	373	453	90,1	nd	**5167**	0,42	6,99	nd	4,67	8,42	**20,5**
2015	B-AC-6	7,72	218	474	532	116	267	31,0	nq	**1646**	0,10	1,70	nd	1,91	1,41	**5,12**
2016	B-AC-7	1,36	260	108	249	127	146	76,7	11,0	**980**	nd	nd	nd	nd	nd	**nd**
2017	B-AC-8	nq	nq	1,46	4,86	2,98	8,57	4,35	nq	**22,2**	0,22	nd	nd	nd	nd	**0,22**
2017	B-AC-9	nq	nd	nd	nq	nd	nq	29,637	nd	**29,6**	nd	nd	nd	nd	nd	**nd**
Purple heron (*Ardea purpurea*)																
2010	B-AP-1	nq	10,2	1,61	0,88	5,68	9,85	nd	6,62	**34,8**	65,2	1,44	nd	nd	nq	**66,7**
2010	B-AP-2	1,3	229	103	228	56	101	18,7	2,68	**741**	21,1	2,20	nd	nd	0,56	**23,8**
2015	B-AP-3	6,81	318	447	751	133	204	29,9	0,19	**1889**	0,24	5,39	nd	0,97	1,32	**7,92**
2016	B-AP-4	0,90	nq	27,3	54,5	17,6	nd	nd	3,65	**104**	nd	nd	nd	nd	nd	**nd**
Western marsh harrier (*Circus aeruginosus*)																
2010	B-CA-1	9,04	168	26,8	162	20,5	222	29,2	6,72	**645**	1,38	8,08	nd	nq	0,89	**10,4**
Black-crowned night heron (*Nycticorax nycticorax*)																
2010	B-NN-1	1,96	134	130	97,4	0,29	165	64,5	10,4	**604**	68,3	3,27	nd	0,29	nd	**71,9**
2010	B-NN-2	1,57	300	171	319	0,21	272	63,1	11,9	**1139**	73,4	12,3	nd	1,66	2,73	**90,1**
2010	B-NN-3	nq	26,0	17,4	28,7	7,69	30,7	nq	4,15	**115**	11,5	3,03	nd	0,07	0,80	**15,4**
2015	B-NN-4	0,38	48,1	nq	nq	nd	28,3	2,25	4,82	**83,8**	2,79	8,52	nd	1,29	nd	**12,6**
2015	B-NN-5	0,36	46,0	60,2	72,1	nd	33,1	10,8	5,47	**228**	2,68	13,1	nd	1,87	2,31	**19,9**
2015	B-NN-6	0,07	21,1	44,1	54,8	15,6	25,9	nd	0,69	**162**	1,67	6,46	nd	nd	nd	**8,13**
2015	B-NN-7	nq	36,4	56,4	85,9	17,5	32,5	nd	nq	**229**	1,45	n. d.	nd	nd	nd	**1,45**
2015	B-NN-8	nd	35,1	72,8	80,1	28,5	61,1	11,0	nq	**289**	0,10	0,75	nd	0,28	0,54	**1,67**
2016	B-NN-9	0,69	nd	18,84	25,2	12,7	19,0	27,5	1,85	**106**	nd	nd	nd	nd	nd	**nd**
2016	B-NN-10	nd	21,1	15,0	2,75	10,2	7,15	nd	1,89	**58,1**	nd	nd	nd	nd	3,00	**3,00**
2016	B-NN-11	0,87	83,4	40,4	83,0	23,5	54,9	nd	nd	**286**	nd	nd	nd	2,44	nd	**2,44**
2016	B-NN-12	nd	33,7	43,8	34,2	38,7	55,2	17,3	1,52	**224**	nd	nd	nd	nd	nd	**nd**
2016	B-NN-13	nd	16,2	17,6	15,3	14,2	27,9	nd	21,5	**113**	nd	nd	nd	nd	nd	**nd**
2016	B-NN-14	nd	67,4	35,4	122	21,5	44,8	nd	nq	**291**	nd	nd	nd	nd	nd	**nd**
2017	B-NN-15	nq	18,7	15,3	18,4	14,7	14,9	11,9	0,47	**94,4**	nq	0,68	nd	nd	nd	**0,68**
2017	B-NN-16	nq	27,7	40,7	26,5	31,3	57,2	34,7	nq	**218**	16,6	9,50	nd	nd	nd	**26,1**
2017	B-NN-17	nq	2,26	3,33	4,90	5,20	10,0	6,57	1,31	**33,6**	nq	nq	nd	nd	nd	**nq**
2017	B-NN-18	nq	21,7	42,9	27,5	32,7	55,6	20,1	1,70	**202**	nq	nd	nd	nd	nq	**nq**
2017	B-NN-19	0,45	24,5	9,98	24,9	11,7	46,3	11,9	3,00	**133**	nd	nd	nd	nd	nd	**nd**

Several studies have reported PBDEs in bird eggs around the world. Five different works reported PBDE levels in heron eggs collected between 1987 and 2014. Custer et al.[1] analyzed PBDEs in 16 great blue heron eggs from three colonies on the Mississippi River (USA) collected during 1993. Concentrations ranged between 4.5 and 1238 ng/g wet weight (ww). Similar levels (70-1377 ng/g ww) were found in great blue heron eggs from Canada during 2001-02[2]. A more recent study[3] showed lower levels in samples from the west coast of North America, with values up to 457 ng/g ww. These three studies carried out in North America presented contamination levels higher than our results, being from 0.67 to 300 ng/g ww (once transformed from lipid base to wet weight). The higher values could be explained by the fact that sample collection was carried out since 1987, time of very high use of PBDE formulations, especially in North America. Two additional works reported PBDE levels in black-crowned night heron samples from Asia during 2004 and 2014[4,5]. Our results (33.6-1139 ng/g lw) are in accordance with those obtained in samples collected in 2004 (184-1040 ng/g lw), while they are higher than those obtained in 2014 (19-150 ng/g lw). Therefore, taking into account that our samples corresponded to 2010-2017, it seems that our study area was most contaminated than the Yangtze River Delta (China)[5].

Since bird egg samples comprising the time period between 2010 and 2017 were analyzed, an evaluation to determine potential temporal trends was carried out. As previously mentioned, the intra-specific variability within samples of the same specie at the same time was high. Moreover, the number of available samples throughout the period studied (2010-17) is not very high, so the conclusions on temporal trends should be taken with caution. Figure 1 shows temporal trends for black-crowned night heron samples, which is the bird species with the largest number of samples (n=19). It seems that PBDE levels declined from the period 2010 (mean value of 619 ng/g lw) to 2015-17 (mean value of 172 ng/g lw). Thus, a trend with the approximately 25% decline in mean PBDE concentrations has been observed during this period of time.

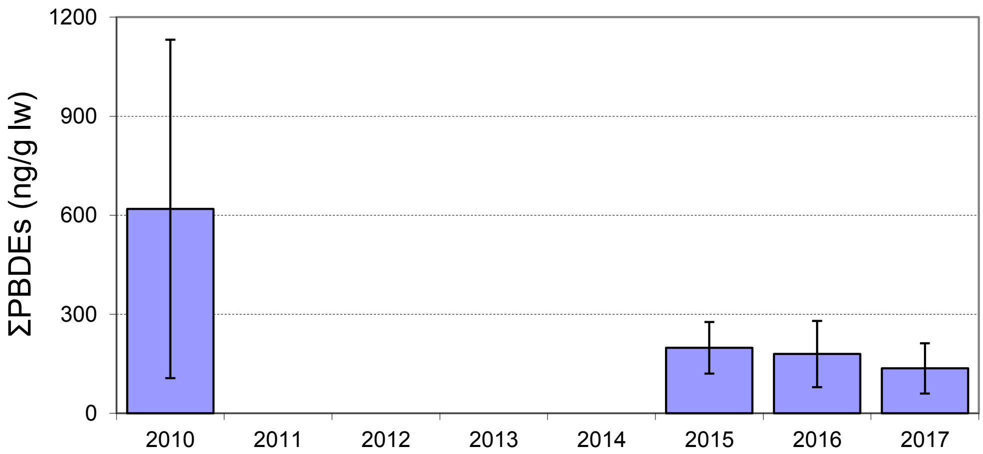

Figure 1. ΣPBDE levels in black-crowned night heron samples grouped by year of collection.

Once concentration levels in our samples were determined, it is necessary to evaluate if these levels are high enough to cause adverse effects in birds. However, there are few studies on the possible toxic effects of PBDEs in birds, and reported LOECs (Table 2). Determined LOECs have been reported to be largely different among studies depending on the exposure systems, specific adverse endpoints and bird species. Each species is likely to be impacted differently by PBDEs due to species-specific differences in genetics, physiology, ecology, life history as well as differences in their environment. Thus LOECs need to be used with caution as estimated thresholds. There is also a threshold level established by the Canadian Federal Environmental Quality Guidelines (FEQGs) at 29 ng/g ww only for Penta-BDEs for bird eggs[6]. In our 33 analyzed eggs, the sum concentrations of BDE-47, -99, -100, and -153 ranged from 1.23 to 282 ng/g ww (Figure 2). Many of the values, specifically 42% of the analyzed samples, were above the Canadian FEQGs level (29 ng/g lw), being the grey heron the species with the highest percentage of samples (78%) with values higher than those recommended. On the other hand, 58% of the samples presented values higher than those associated with hepatic oxidative stress, marginal lipid peroxidation and changes in glutathione metabolism in American kestrel (15.6 ng/g ww)[8]. These results suggest that, even though PBDEs were internationally banned in 2009, the studied bird species were actually still exposed to PBDE concentrations that are associated with possible ecological hazards.

Acknowledgements

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Table 2. Lowest observed effect concentrations (LOECs) for PBDEs in birds.

	Species	Observed adverse effects	LOEC (ng/g ww)	Reference
BDE-47, -99, -100 and -153	American kestrel (*Falco sparverius*)	Spleen somatic index	86.1	7
BDE-47, -99, -100 and -153	American kestrel (*Falco sparverius*)	Hepatic oxidative stress, marginal lipid peroxidation and changes in glutathione metabolism	15.6	8
BDE-47, -99, -100 and -153	American kestrel (*Falco sparverius*)	Larger growth, affecting bone structure and energetic cost	1500	9
Penta-BDE DE-71	American kestrel (*Falco sparverius*)	Copulation frequency, sperm numbers, time spent in nest boxes decrease	288.6	10,11
PBDEs*	Ospreys	Reduce productive performance	1000	12

* Sum of BDE-17, -28,- 47, -49, -66, -85, -99, -100, -138, -153, -154, -183, -190 and -209

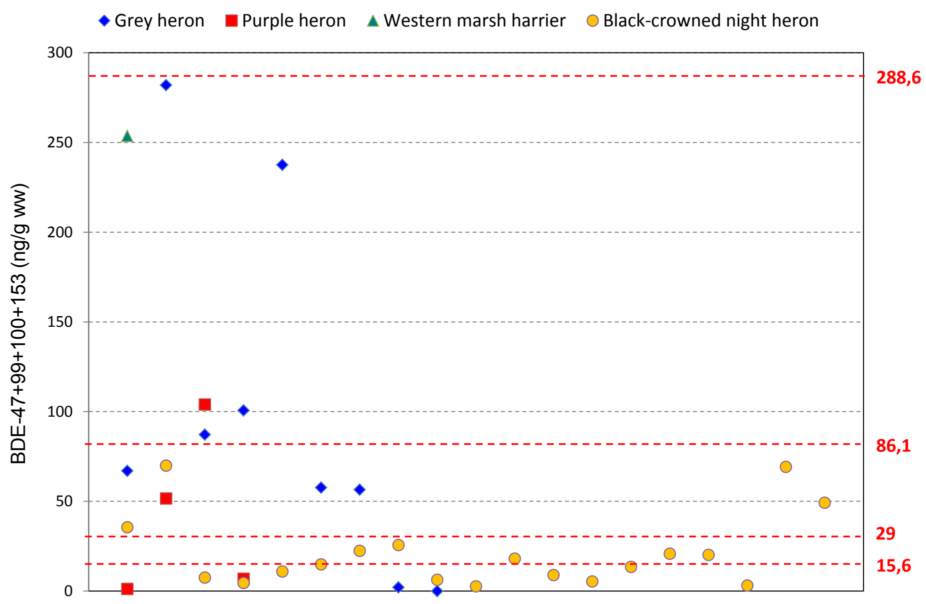

Figure 2. Comparison between PBDE levels and LOEC values.

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