EMERGING HALOGENATED FLAME RETARDANTS IN PEREGRINE FALCON (*Falco peregrinus*) EGGS FROM CANADA AND SPAIN.

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Introduction

Halogenated flame retardants (HFRs), are a structurally diverse group of chemicals generally brominated or chlorinated. These compounds are added to or reacted with polymers, textiles, and electronic circuitry to reduce the risk of fire. The most utilized are the brominated flame retardants (BFRs). Thus, more than 75 different bromine-based compounds are registered in commerce, including polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA). Few years ago, polybrominated diphenyl ethers (PBDEs) have attracted the most attention, due to their ubiquitous occurrence and relatively high levels in the environment. Due to their toxicological effect, the production and use of BDE formulations are banned in Europe and, Penta- and Octa-BDE formulations have been banned in North America. However, there are stocks of all PBDEs from products in service and waste. Therefore, in response to the demand for fire safety regulation the industry has introduced several new HFR[1].

HBCD is a mixture of three diastereoisomers α-, β- and γ- isomer[2]. The physical-chemical properties of HBCD are similar to some PBDEs and other persistent organic pollutants (POPs). HBCD have been detected worldwide[3]. In addition to HBCDs, new HFR such as pentabromoethylbenzene (PBEB), hexabromobenzene (HBB), 2,2',4,4',5,5'-hexabromobiphenyl (BB-153), 1,2-Bis(2,4,6-Tribromophenoxy)ethane (BTBPE) and decabromodiphenylethane (DBDPE) have been measured in North America and Europe. Little or no toxicological data is available for wildlife or humans for these HFRs in the open literature.

On the other hand, while policy makers consider further restrictions on these currently used BFRs, Dechlorane Plus (DP), a chlorinated flame retardant ($C_{18}H_{12}Cl_{12}$) manufactured for over 40 years, has only recently been reported in the environment and biota. DP is only produced in Oxychem (Niagara Falls, NY) with an estimated production that ranged between 1 and 10 million pounds/year since 1986. However, DP is sold worldwide, including Europe and Asia[4].

Johansson et al. (2009)[5] recently reported accumulation of some flame retardants as PBDEs, BB-153 and HBCD in Swedish peregrine falcon eggs. Peregrine falcons feed primarily on other medium-sized birds, and most populations of the species were previously endangered in the northern hemisphere because of the bioaccumulation of high concentrations of several organochlorine pesticides and mercury, which affected both reproduction and survival. After the ban of several pesticides, polychlorinated biphenyls, and mercury in many countries of the northern hemisphere in the latest 1970s, peregrine populations have been recovered[6,7]. Peregrine are useful sentinel species for monitoring environmental organic contaminants because, as bird-eating raptors, they are at the top of the food chains, consuming prey from the aquatic and/or terrestrial environments, including migratory birds[8].

The aim of this study is to investigate the occurrence of halogenated norbornenes (mirex, DP, DP602, DP603, DP604 and DP monoadduct (DPMA)), hexabromocyclododecane (HBCDs), hexabromobenzene (HBB),

2,2',4,4',5,5'-hexabromobiphenyl (BB-153), 1,2-Bis(2,4,6-Tribromophenoxy)ethane (BTBPE), hexachlorocyclopenthenyl-dibromocycloctane (HCBCO), bis (2-ethyl-1 hexyl)tetrabromophenyl ether (BEHTBP), octabromotrimethylphenylindane (OBIND), decabromodiphenylethane (DBDPE) and polybrominated diphenyl ethers (PBDEs) in Peregrine falcon eggs from Canada and Spain.

Materials and methods

Study Area. Peregrine falcon eggs were collected in Canada (n=12) and Spain (n=13) between 2007-2009 and 2003-2006, respectively. Collected egg samples were classified according to the peregrine diet: terrestrial (S1-S5 and C1-C7, C10-C12) or aquatic (S6-S13, C8 and C9).

Sample preparation. Before extraction, sampled eggs were lyophilized and homogenized. 3-5 g dry weight (dw) of sample was Soxhlet extracted using 250 mL of dichloromethane for one night. After extraction, the crude extracts were concentrated to around 2 mL, and then subjected to a purification step by Gel Permeation Chromatography (GPC). 150 mL of DCM:Hexane (1:1 v/v) were eluted with the compounds of interest. Then, the extracts were concentrated to 3 mL and transferred to a silica gel column. Different solvents were used in order to obtain three fractions with the compounds of interest. Samples were finally concentrated to incipient dryness and re-dissolved with 500 µL of solvent prior to the instrumental analysis by LC-MS/MS or HRGC/LRMS.

HRGC/LRMS. PBDEs (3, 15, 28, 47, 77, 99, 100, 126, 153, 154, 169, 183, 197, 205, 207 and 209), mirex, DP, DP602, DP603, DP604, HBB, BB-153, BTBPE, HCBCO, BEHTBP, OBIND and DBDPE 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 Technologies, Palo Alto, CA, USA) operated in EI positive. The chromatographic separation was performed on a DB-5HT 15m x 0.25 mm x 0.10 um.

LC-MS/MS. The analysis of isomeric and enantiomeric HBCD was performed using a LC system Agilent HP 1200 binary pump SL (Palo Alto, CA, USA) fitted with a hybrid triple quadrupole/linear ion trap AB SCIEX Triple Quad™ 5500 system (Applied Biosystems, Foster City, CA, USA) instrument equipped with an electrospray (ESI) Turbospray interface, and working in negative ionization mode. In the analysis of isomeric-HBCD, a Zorbax Eclipse C_{18} column (2.1 mm x 100mm, 3.5 µm) supplied by Agilent was used. A chiral chromatographic column, Nucleodex β-PM (4.0 x 200 mm x 5µm), was used to afford the enantiomer-specific determination.

Results and discussion

In Table 1 the concentration of HBB, BB-153, HCDBCO, BEHTBP, OBIND, DBDPE, BTBPE, ΣHBCDs and ΣPBDEs were summarized. In general, the values of these compounds were higher in the samples from Canada. Of the 25 egg samples, ΣHBCDs were detected in the 80% of samples at concentrations between 1.0 and 14 617 ng/g (lw). All these values were congruent with the results found in samples from Sweden and Greenland[5,9]. In general, α-HBCD was the predominant diastereoisomer, as has been reported in bird egg from other locations[10]. Concentrations of ΣPBDEs ranged from 162 to 37 517 ng/g lw in the 25 eggs, and were approximately the double of ΣHBCDs values. BB-153 was detected in all samples at concentrations between 3.6 and 1271 ng/g lw. In the case of HBB, HCBCO, BEHTBP and OBIND concentrations from nd-7.2, nd-23, nd-4.5 and nd-29 respectively, were found. To date, no data has been reported about HCDBCO and BEHTBP in biota, just in house air and dust[11,12]. The presence of OBIND was first reported in white stork (*Ciconia ciconia)* eggs from Spain[13].

Collected egg samples were classified according to the peregrine diet: terrestrial or aquatic. In the case of samples from Spain, lower values of ΣHBCDs, ΣPBDEs and BB-153 had been noticed in eggs from peregrines with terrestrial diet.

Concentrations of mirex, DP602, DP603, DP604 as well as syn- and anti-DP stereoisomers, in peregrines egg are presented in Table 2. DP602, DP603 and DP604 were detected in more than 96% of samples, it is important to note that because of their high chlorination these compounds are persistent and bioaccumulative in the environmen[14]. The

values of these compounds were higher in samples from Canada. In the case of peregrines from Spain with an aquatic diet (Spain-A) higher levels of DP602 and DP603 were observed compared to terrestrials.
Although mirex was banned in the '80s, it was still detected in all egg samples, and its concentration varied from 2.4 to 1353 ng/g lw. DP was also detected in all samples, and values of total DP were ranged from 0.3 to 209 nd/d lw. A previous work[4] reported total DP values lower than 15 ng/g ww in gull eggs from the Laurentian Great Lakes. The concentrations of the present study are in the same range, with values not higher than 13 ng/g ww.

DP may have been subject to stereoselective processes in the environment prior to uptake or via biochemical processes. Thus, the anti-DP fraction (*f*anti) is calculated as the concentration of anti-DP divided by the concentration of total DP. In Table 2 median values of *f*anti are presented. Regarding different bird diet, samples from Spain with a terrestrial diet (Spain-T) *f*anti values (0.74) are similar with the *f*anti of the technical DP presented by Gauthier et al. (0.75-0.77)[4], suggesting that there is no stereoselective enrichment of syn-DP or anti-DP isomer in the peregrine eggs from Spain with terrestrial diet. However, in Spain-A samples the *f*anti (0.69) is lower, indicating a little enrichment of syn-DP.
In the case of Canadian samples, less *f*anti were observed. The median were 0.63 and 0.56 for peregrines with aquatic and terrestrial diet, respectively. These results showed a stereoselective enrichment of syn-DP in the sampled egg. Different result obtained by Gauthier and Letcher[4] indicated no enrichment of syn- or anti-DP in herring gull eggs. In contrast, for smelt and alewife from Lake Ontario mean *f*anti about 0.53 were reported[15].

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References

1. Verreault J, Gebbink WA, Gauthier LT, Gabrielsen GW, Letcher RJ. *Environ. Sci. Technol.* 2007. 41:4925.
2. Guerra P, De La Torre A, Martinez MA, Eljarrat E, Barcelo D. *Rapid Commun. Mass Spectrom.* 2008. 22:916.
3. Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR, De Boer J. *Environ. Sci. Technol.*2006. 12:3679.
4. Gauthier LT, Letcher RJ. *Chemosphere*. 2009, 75:115.
5. Johansson AK, Sellstrom U, Lindberg P, Bignert A, De Wit CA. *Environ. Toxicol. Chem*. 2009. 28: 4925.
6. Fernie KJ, Letcher RJ. *Environ. Sci. Technol.* 2010. 44: 3520.
7. Merino R, Bordajandi LR, Abad E, Rivera J, Jimenez B. *Environ. Toxicol. Chem*. 2005. 24:2088.
8. Park JS, Holden A, Chu V, Kim M, Rhee A, Patel P, Shi Y, Linthicum J, Walton B, McKeown K, Jewell NP, Hooper K. *Environ. Sci. Technol.* 2009. 43: 8744.
9. Vorkamp K, Thomsen M, Falk K, Leslie H, Moller S, Sorensen PB. *Environ. Sci. Technol.* 2005. 21: 8199.
10. Janak K, Sellstrom U, Johansson AK, Becher G, de Wit CA, Lindberg P, Helander B. *Chemosphere*. 2008, 73: S193.
11. Lam JCW, Lau RKF, Murphy MB, Lam PKS. *Environ. Sci. Technol.* 2009. 43: 6944.
12. Zhu J, Hou Y, Feng YL, Shoeib M, Harner T. *Environ. Sci. Technol.* 2008. 42: 386.
13. Muñoz-Arnanz J, Sáez M, Pacepavicius G, Jiménez B, Alaee M. *BFR 2010*. Kyoto, Japan. 7-9 April 2010. http://bfr2010.com/abstract-download/2010/
14. Shen L, Reiner EJ, Macpherson KA, Kolic TM, Sverko E, Helm PA, Bhavsar SP, Brindle ID, Marvin CH. *Environ. Sci. Technol.* 2010. 44:760.
15. Tomy GT, Pleskach K, Ismail N, Whittle DM, Helm PA, Sverko ED, Zaruk D, Marvin CH. *Environ. Sci. Technol.* 2007. 41: 2249.

Table 1. Summary statistics for HBB, BB-153, HCDBCO, BEHTBP, OBIND, DBDPE, ΣHBCDs and ΣPBDEs in falcon eggs from Canada and Spain.

		Compound								
		HBB	**BB-153**	**HCDBCO**	**BEHTBP**	**OBIND**	**DBDPE**	**BTBPE**	**Σ HBCDs**	**Σ PBDEs**
Spain-T	**Positive samples**	3	5	0	0	2	0	0	2	5
(n=5)	**Range (ng/g, lw)**	nd-3.1	3.6-11			nd-0.84			nd-340	162-3595
	Median (ng/g, lw)	1.5	8.0			0.53			0.40	227
Spain-A	**Positive samples**	4	8	0	1	3	0	1	7	8
(n=8)	**Range (ng/g, lw)**	nd-0.99	11-67		nd-1.2	nd-1.0		nd-7.4	nd-1598	710-5304
	Median (ng/g, lw)	0.35	23		1.1	0.27		5	271	1210
Canada-T	**Positive samples**	6	10	2	4	4	1	0	9	10
(n=10)	**Range (ng/g, lw)**	nd-7.2	12-1271	17-23	nd-4.5	nd-29	nd-8.2		nd-14617	533-37517
	Median (ng/g, lw)	4.8	154	3.5	0.5	0.27	6.7		2772	4287
Canada-A	**Positive samples**	0	2	1	0	0	0	1	2	2
(n=2)	**Range (ng/g, lw)**		7-450	nd-19				nd-14	325-3340	1503-7339
	Median (ng/g, lw)		229	13				8	1847	4421
	LOD (ng/g, lw)	0.09	0.50	2.2	0.60	0.20	1.1	0.67	0.12	0.11
	LOQ (ng/g, lw)	0.31	1.7	6.9	1.0	0.53	5.2	2.2	0.41	0.42

LOD: limit of detection; LOQ: limit of quantification; nd: not detected; T: terrestrial; A: aquatic, HBB: hexabromobenzene, BB-153: 2,2',4,4',5,5'-hexabromobiphenyl, HCBCO: hexachlorocyclopenthenyl-dibromocycloctane, BEHTBP: Bis (2-ethyl-1 hexyl)tetrabromophenyl ether, OBIND: octabromotrimethylphenylindane, DBDPE: decabromodiphenylethane, BTBPE: 1,2-Bis(2,4,6-Tribromophenoxy)ethane, Σ HBCDs: total HBCD, Σ PBDEs: total PBDEs.

Table 2. Summary statistics for DPMA, mirex, DP602, DP603, DP604, syn-DP and anti-DP in falcon eggs from Canada and Spain.

Sample Type		Compound							
		DPMA	**mirex**	**DP602**	**DP603**	**DP 604**	**syn-DP**	**anti-DP**	***f*anti***
Spain-T	**Positive samples**	5	5	4	5	4	4	5	
(n=5)	**Range (ng/g, lw)**	1.7-37	2.4-17	nd-15	1.5-6.2	nq-0.35	nd-1.4	0.3-2.2	0.62-0.75
	Median (ng/g, lw)	2.5	14	4.8	2.4	0.32	0.20	0.60	0.74
Spain-A	**Positive samples**	7	8	8	8	5	6	8	
(n=8)	**Range (ng/g, lw)**	nd-469	9.2-78	nd-25	3.0-7.5	nd-0.32	nd-2.6	0.8-12	0.65-0.78
	Median (ng/g, lw)	51	29	15	5.2	0.31	0.54	2.0	0.69
Canada-T	**Positive samples**	10	10	10	10	10	8	10	
(n=10)	**Range (ng/g, lw)**	1.2-1656	425-1353	44-211	12-220	1.4-9.8	2.9-89	4.6-120	0.41-0.69
	Median (ng/g, lw)	62	744	71	52	4	14	24	0.63
Canada-A	**Positive samples**	2	2	2	2	2	2	2	
(n=2)	**Range (ng/g, lw)**	3.8-218	94-1158	7.2-104	5.3-29	1.3-3.5	2.5-59	3.8-63	0.52-0.6
	Median (ng/g, lw)	111	626	55	17	2	31	34	0.56
	LOD (ng/g, lw)	0.05	0.01	0.03	0.01	0.03	0.01	0.004	0.40
	LOQ (ng/g, lw)	0.15	0.04	0.09	0.02	0.09	0.02	0.01	0.38

LOD: limit of detection; LOQ: limit of quantification; nd: not detected; T: terrestrial; A: aquatic
(*): anti-DP fraction (fanti) was calculated as the concentration of anti-DP divided by the concentration of total DP