BROMINATED FLAME RETARDANTS AND HYDROXYLATED AND METHOXYLATED ORGANOHALOGEN CONTAMINANTS IN THE PLASMA OF NORTH AMERICAN WEST COAST BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*)

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Introduction

Brominated flame retardants, including polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD) and especially polybrominated diphenyl ethers (PBDEs) are of environmental concern and currently under intense study in wildlife and humans. Similar to polychlorinated biphenyls (PCBs), the physico-chemical properties of PBDEs render them persistent and bioaccumulative [1]. Temporal studies have shown exponentially increasing PBDE levels over the last 20 years in the eggs of double-crested cormorants (*Phalacrocorax auritus*) and great blue herons (*Ardea herodias*), two species of fish-eating birds in southwestern British Columbia (BC), Canada [2]. PBDEs have been determined in Great Lakes bald eagle (*Haliaeetus leucocephalus*) nestling plasma [3], but there are no reports of brominated flame retardants in any raptor species or population from the west coast of North America.

Although the toxicokinetics of PCBs and particularly PBDEs are not well understood in birds [4], ostensible metabolites of both organohalogen classes have been reported in the tissues of a limited number of bird species. For instance, several hydroxy- (OH-) PCB congeners were recently quantified in Faroe Island fulmar (*Fulmarus alacialis*) eggs [5] and in glaucous gull (*Larus hyperboreus*) eggs and adult plasma from the Norwegian Arctic [6]. A few OH-PBDE and/or methoxy- (MeO-) PBDE congeners have been reported in the same glaucous gulls [7], as well as in Baltic white-tailed sea eagle plasma [8]. The presence of OH-PCBs as residues in the plasma of birds and other wildlife is more than likely due to cytochrome P450-mediated PCB metabolism (although bioaccumulation is a remote possibility) and subsequent retention of selected congeners through interactions with thyroid hormone transport proteins [9]. Similar oxidative biotransformation of PBDEs has been demonstrated in rats and fish [4], however MeO-PBDEs and some OH-PBDE congeners are more likely to be bioaccumulated natural products produced by marine organisms such as sponges and algae [10,11].

The bald eagle is an ideal sentinel species for monitoring the levels and effects of organohalogen contaminant exposure in the North American environment [12]. As is the case for other predatory birds occupying top trophic positions, many bald eagle populations have exhibited toxicological effects, such as eggshell thinning and mortality, associated with contaminant exposure (e.g., PCBs) [13]. Populations from the southern coastal Californian Channel Islands were extirpated in the 1960s due to high organochlorine exposures [14], but were reintroduced to the island of Santa Catalina more than 20 years ago. Further up the west coast, populations in southwestern BC have not decreased to the same extent but some have exhibited reduced nesting success possibly related to contaminant exposures [15]. Bald eagles from the Fort St. James area of northern BC are spatially removed from concentrated urban and industrial zones and therefore the region is considered to be a site of low organochlorine exposure.

The present study aimed to identify and quantify brominated flame retardants (PBDEs, PBBs and HBCD) as well as OH-PCBs, OH-PBDEs, MeO-PBDEs and 4-OH-heptachlorostyrene (4-OH-HpCS) in North American west coast

nestling bald eagle plasma. Spatial trends, including the concentrations and congener patterns, of these contaminants among four southwestern coastal BC populations, one northern BC reference populations and one southern Californian population were assessed.

Materials and Methods

Chosen to reflect variation in contaminant exposure due to differing current and historical industrial activities, blood samples were collected from nestling bald eagles at five BC sites and one in California (Figure 1). The BC locations were Fort St. James (FSJ; northern BC; *n* = 4), Barkley Sound (BS; southwest Vancouver Island; *n* = 3), Nanaimo-Crofton (N-C; southeast Vancouver Island; *n* = 7), Delta-Richmond (D-R; lower Fraser Valley; *n* = 6) and Abbotsford-Chilliwack (A-C; central Fraser Valley; *n* = 6). In California, samples were taken from Santa Catalina Island (SCI; southern California; *n* = 3) and pooled to obtain sufficient sample size for analysis. After isolation of the plasma, chemical fractionation and clean-up were carried out according to previously published methods [16]. Plasma samples were spiked with appropriate internal standards (BDE30, 2'-OH-BDE28 and a mixture of 12 ^{13}C-labelled OH-PCBs) and extracted into a neutral fraction (analyzed for PBDEs, PBBs, total-HBCD and MeO-PBDEs) and a phenolic fraction (analyzed for 4-OH-HpCS, OH-PCBs and OH-PBDEs, derivatized to their MeO-analogues). Recoveries were 90%, 84% and 53% for PBDEs (and MeO-PBDEs), OH-PCBs and OH-PBDEs, respectively. Separation and quantification of analytes were performed by GC/MS in the electron capture, negative ionization (ECNI) and selected ion monitoring (SIM) modes [7]. As described elsewhere [17], and used for the purpose of comparison in this study, PCBs were also extracted and analyzed.

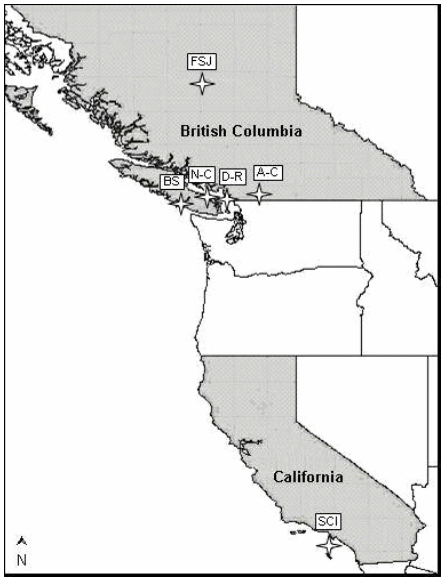

Figure 1. Map showing the locations of nestling bald eagle sampling sites in British Columbia and California. Sites are as follows: FSJ = Fort St. James, BS = Barkley Sound, N-C = Nanaimo-Crofton, D-R = Delta-Richmond, A-C = Abbotsford-Chilliwack and SCI = Santa Catalina Island.

Results and Discussion

The plasma ΣPBDE concentrations were similar among the southwestern BC nestling bald eagle populations (Figure 2). Comparable ΣPBDE levels, at 7.9 ng/g wet weight (w.w.), were reported previously in bald eaglet plasma samples from the Laurentian Great Lakes region [3]. Significant variation in the mean ΣPBDE levels among BC nestlings (ANOVA: $F_{4,21} = 0.402$, $p < 0.001$) was attributed to higher concentrations in all southwestern BC populations than in nestlings collected from the FSJ reference site. Although this may be related to the proximity of the southwestern BC eaglets to urban and industrial areas, spatial variation in PBDE contamination may be more related to dietary differences between populations, as has been previously suggested to be the case for PCBs [18]. In eaglets from SCI, ΣPBDE levels were from 4- to 77-fold higher than in BC nestlings. In contrast to BC eaglets and unlike most current wildlife findings [1], nestlings from SCI demonstrated higher (2.5-fold) ΣPBDE than ΣPCB concentrations (Figure 2). Mainly tetra- and penta-brominated PBDE congeners were detected in BC eaglets. Congener profiles were similar among the BC populations; BDE47 accounted for around half of the ΣPBDE, BDE99 and BDE100 represented around one quarter each and the remainder consisted of BDE153. The pattern was comparable in SCI nestlings, except that low levels of BDE138, BDE154 (co-eluted with BB153) and BDE183 were also detected. This finding may suggest differences in source patterns between BC and SCI nestlings or the closer proximity of SCI to major sources of release (e.g., the industrial and heavily urbanized areas of Los Angeles). The fully brominated BDE209 was not detected in SCI nor in BC eaglets, possibly due to the relatively lower

bioavailability and/or higher (photolytic or metabolic) debromination potential of this congener [1]. BB101 was found at similar concentrations (mean 0.36 to 0.73 ng/g w.w.) in all nestlings located in southwestern BC, at levels from 5- to 15-fold lower than ΣPBDE levels. However, this PBB was not found in nestlings from FSJ or SCI. BB153 co-eluted with BDE154, but a combined peak was only found in SCI plasmas and at <1% of ΣPBDE levels. Although HBCD was recently reported in Norwegian glaucous gulls [7], total (α)-HBCD was not detected in any North American eaglet plasma analyzed.

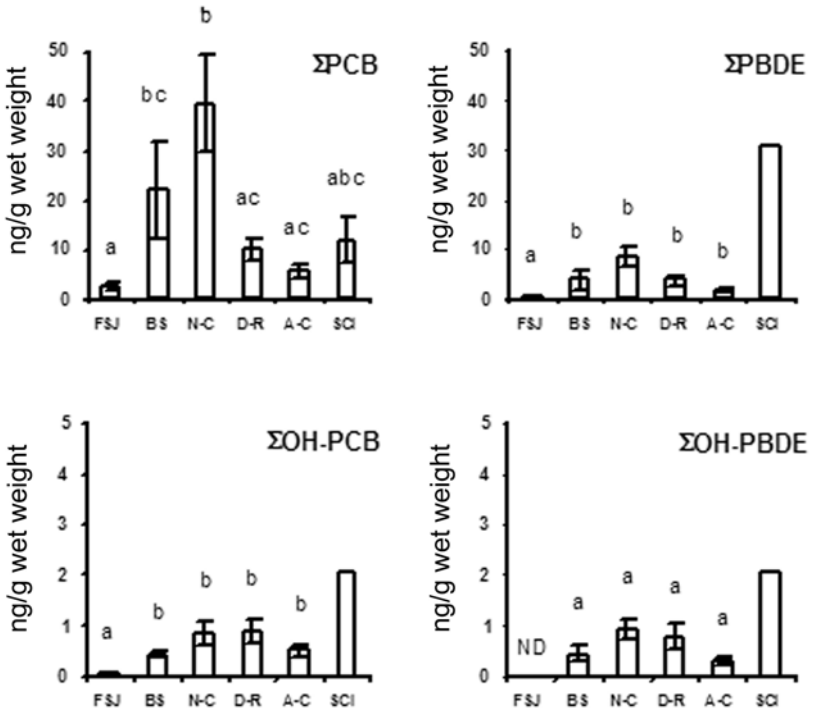

Figure 2. Arithmetic mean concentrations (± S.E.) of organohalogen contaminants in nestling bald eagles from British Columbia and southern California sites. Means that do not share the same lower case letter were significantly different ($p < 0.05$). Y-axes are not drawn to the same scale for all panels. Site abbreviations are as indicated in Figure 1.

OH-PCBs were detected in nestlings from all populations. The ΣOH-PCB concentrations were significantly higher in southwestern BC eaglets than in FSJ eaglets (Table 1). Although mean ΣPCB levels in SCI nestlings were not significantly different than in any of the BC populations, the eaglets from SCI contained higher ΣOH-PCB levels than all BC populations. This finding may be related to exposure induced biotransformation potential towards PCBs in the organochlorine-contaminated SCI eaglets and/or their mothers [9]. Only highly chlorinated (hexa- to nona-Cl) congeners were found in bald eaglets from all populations. The major congeners detected in BC nestlings, 4-OH-CB187 and 4'-OH-CB202, accounted for between 65% and 100% of the ΣOH-PCB. The latter congener was also dominant in SCI plasma in conjunction with the 3'-OH-CB138 and 4-OH-CB146. In the blood or plasma of four other bird species studied, 4-OH-CB187 and 4-OH-CB146 were identified as the major congeners [6,8,9]. The greater proportion of highly chlorinated OH-PCBs in BC bald eagles may not be due to metabolism alone and is possibly also related to dietary uptake; however, the importance of biotransformation versus bioaccumulation for OH-PCBs in birds requires further investigation. 4-OH-HpCS, an ostensible metabolite of octachlorostyrene (OCS), was also detected in eaglets from all populations (Table 1). Chlorinated phenolic contaminants contributed considerably to the overall level of organohalogen contamination in these eaglet plasmas.

The levels of ΣOH-PBDE were similar to the ΣOH-PCB concentrations found for all populations (Figure 2), but MeO-PBDEs were not detected in any population. The mean ΣOH-PBDE levels were similar among southwestern BC populations and were all significantly higher than in FSJ nestlings (> 50% of samples below the MLOQ). In contrast to OH-PCBs, the OH-PBDE congener patterns were rather simple and distinctly different between BC and SCI nestlings. Two tetra-brominated congeners, 6'-OH-BDE49 and 6-OH-BDE47 were found in BC eaglets, but only 4'-OH-BDE49 was found in the pooled SCI sample. The level of 4'-OH-BDE49 was at least 2-fold higher than the

sum of 6'-OH-BDE49 and 6-OH-BDE47 in all BC populations. The *ortho*-OH-substituted OH-PBDEs determined in BC birds are theoretical metabolites of anthropogenic PBDEs [4], but are more than likely bioaccumulated natural products [11]. In contrast, the *para*-substituted congener found in SCI is probably a metabolite of BDE47, formed via an NIH-shift mechanism similar to OH-PCB formation from PCBs [9]. Given the variety of organohalogens to which west coast North American bald eagles are exposed, further studies should investigate potential effects of these contaminants, such as interactions with thyroid hormones and vitamin A levels.

Table 1. Arithmetic mean ± SE and range of OH-PBDE, MeO-PBDE, OH-PCB and 4-OH-HpCS concentrations (ng/g wet weight) in nestling bald eagle plasma samples from British Columbia and southern California sites.

	Fort St. James (n = 4)		Barkley Sound (n = 3)		Nanaimo-Crofton (n = 7)		Delta-Richmond (n = 6)		Abbotsford-Chilliwack (n = 6)		Santa Catalina Island (n = 3)*	
Analyte	Mean ± SE (range)	%n > MLOQ	Mean ± SE (range)	%n > MLOQ	Mean ± SE (range)	%n > MLOQ	Mean ± SE (range)	%n > MLOQ	Mean ± SE (range)	%n > MLOQ	Mean ± SE (range)	%n > MLOQ
6'-OH-BDE49	<0.01	0	(<0.01 – 0.40)	33	0.54 ± 0.17 (<0.01 – 1.17)	71	0.46 ± 0.20 (<0.01 – 1.24)	66	<0.01	0	<0.01	0
6-OH-BDE47	(<0.01 – 0.47)	25	0.32 ± 0.01 (0.30 – 0.35)	100	0.38 ± 0.13 (<0.01 – 1.03)	71	0.31 ± 0.11 (<0.01 – 0.61)	66	0.31 ± 0.10 (0.14 – 0.64)	83	<0.01	0
4'-OH-BDE49	<0.01	0	<0.01	0	<0.01	0	<0.01	0	<0.01	0	2.10	100
ΣOH-PBDE	(NQ – 0.47)	25	0.46 ± 0.15 (0.30 – 0.75)	100	0.92 ± 0.20 (NQ– 1.51)	86	0.77 ± 0.27 (0.18 – 1.85)	100	0.31 ± 0.10 (NQ – 0.64)	83	2.10	100
ΣMeO-PBDE	NQ	0	NQ	0	NQ	0	NQ	0	NQ	0	NQ	0
ΣOH-PCB	0.04 ± 0.02 (0.01 – 0.09)	100	0.44 ± 0.06 (0.37 – 0.56)	100	0.83 ± 0.24 (0.47 – 2.28)	100	0.87 ± 0.24 (0.35 – 1.98)	100	0.51 ± 0.12 (0.37 – 1.02)	100	2.03	100
4-OH-HpCS	0.01 ± 0.01 (0.01 – 0.02)	100	0.03 ± 0.01 (0.02 – 0.03)	100	0.03 ± 0.01 (0.01 – 0.05)	100	0.06 ± 0.01 (0.02 – 0.08)	100	0.05 ± 0.01 (0.01 – 0.09)	100	0.67	100

*The 3 Santa Catalina Island samples were pooled prior to analysis.

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References

1. Birnbaum LS, Staskal DF. *Environ Health Perspect* 2004;112:9-17.
2. Elliott JE, Wilson LK, Wakeford B. *Environ Sci Technol* 2005;39:5584-5591.
3. Dykstra CR, Meyer MW, Rasmussen PW, Warnke DK. *J Great Lakes Res* 2005;31:227-235.
4. Hakk H, Letcher RJ. *Environ Int* 2003;29:801-828.
5. Fangström B, Athanasiadou M, Athanassiadis I, Weihe P, Bergman Å. *Ambio* 2005;34:184-187.
6. Verreault J, Letcher RJ, Muir DCG, Chu SG, Gebbink WA, Gabrielsen GW. *Environ Toxicol Chem* 2005;24:2486-2499.
7. Verreault J, Gabrielsen GV, Chu SG, Muir DCG, Andersen M, Hamaed A, Letcher RJ. *Environ Sci Technol* 2005;39:6021-6028.
8. Olsson A, Ceder K, Bergman Å, Helander B. *Environ Sci Technol* 2000;34:2733-2740.
9. Letcher RJ, Klasson-Wehler E, Bergman Å. In *The Handbook of Environmental Chemistry*, Paasivirta J. (ed.), Springer-Verlag, Heidelberg, 2000:315.
10. Malmvärn A, Marsh G, Kautsky L, Athanasiadou M, Bergman Å, Asplund L. *Environ Sci Technol* 2005;39:2990-2997.
11. Teuten EL, Xu L, Reddy CM. *Science* 2005;307:917-920.
12. Bowerman WW, Best DA, Grubb TG, Sikarskie JG, Giesy JP. *Chemosphere* 2000;41:1569-1574.
13. Elliott JE, Harris ML. *Rev Toxicol* 2001/2002;4:1-60.
14. Kiff LF. In *The California Islands: Proceedings of a Multidisciplinary Symposium*, Power DM. (ed.), Santa Barbara Museum of Natural History, Santa Barbara, 1980:651.
15. Gill CE, Elliott JE. *Ecotoxicology* 2003;12:95-111.
16. Hovander L, Athanasiadou M, Asplund L, Jensen S, Klasson-Wehler E. *Anal Toxicol* 2000;24:696-703.
17. Cesh LS. MSc Thesis, Department of Biological Sciences, Simon Fraser University, Burnaby, Canada, 2005.
18. Elliott JE, Norstrom RJ, Smith GEJ. *Arch Environ Contam Toxicol* 1996;31:354-367.