POLYHALOGENATED AROMATIC HYDROCARBONS AND METABOLITES: RELATION TO CIRCULATING THYROID HORMONE AND RETINOL IN NESTLING BALD EAGLES (HALIAEETUS LEUCOCEPHALUS)

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Abstract—Polyhalogenated aromatic hydrocarbons are global contaminants that are often considered to be endocrine disruptors and include 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (p,p′-DDE), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). The present study examined these compounds and their hydroxylated metabolites or analogues and relationships with circulating thyroid hormones and retinols in plasma from nestling and adult bald eagles in British Columbia, Canada, and California, USA. We also compared our results with published data. Thyroxine (T4) decreased with ∑PCB and CB153 in nestling bald eagles, which was congruent with results from nine of 14 other published avian laboratory and field studies. Free thyroid hormone levels also decreased with CB-153 and hydroxylated PCBs (OH-PCBs). Retinol increased with CB118 and CB180 in nestling eagles, decreased with OH-PCBs in a subset of nestlings, and decreased in 7 of 12 PCB published studies. Thyroxine decreased with p,p′-DDE for nestlings and with data reported in one of five other published studies. In our samples, plasma retinol, triiodothyronine (T3), and T4 were independent of ∑PBDEs, whereas ∑OH-PBDEs were weakly but significantly correlated with increases in T3 and retinol. Adult bald eagles showed no relationship between contaminants and thyroid hormones, which is consistent with other studies of long-lived birds,

metabolism, binding, and excretion of thyroid hormones [1,2,19,20]. Thyroid hormones are essential for normal growth and development; any disruption in their levels could have significant consequences especially for developing young [3,19,21,22]. Retinol production, which is influenced by thyroid function, has also been affected by PHAH contamination in birds [23]. Retinol is needed for vision, bone growth, reproduction, and immune system function and acts as an antioxidant.

Hydroxylated polychlorinated biphenyls (OH-PCBs) and to a lesser extent OH-PBDEs are being reported with increasing frequency in (mainly the blood of) wildlife, including birds, and they may also impact the thyroid system [24]. A recent laboratory study showed that the hydroxylated analogues of PBDEs (OH-PBDEs) and metabolites of PCBs (OH-PCBs) act as thyroid hormone-like agents, with *meta*- or *para*-hydroxyl groups and adjacent dibromo substitution favoring thyroid hormonal activity [25]. Similarly, a recent study with herring gulls found that OH-PBDEs and OH-PCBs had more potent competitive binding to both triiodothyronine (T3) and thyroxine (T4) than nonhydroxyl PBDE and PCB analogues, with OH-PBDEs being more effective competitors to both T3 and T4 than the OH-PCBs [26]. However, relatively few studies have investigated possible effects of PBDEs and OH-PBDEs on thyroid hormones of wild birds.

The specific objective of the present study was to examine potential effects of PHAHs on the thyroid hormones T3 and T4, as well as retinol, in nestling and adult bald eagles from British Columbia and California. Bald eagles are top predators in the aquatic environment [27,28] and also feed in land where they may be exposed to continued high levels of PBDEs, PCBs, and other compounds [29,30], so they may be among the most useful species for examining health effects for these compounds in the wild [31,32]. We use an information theoretic approach to determine which models best explain relations between measured contaminants and retinol and thyroid hormone levels. We

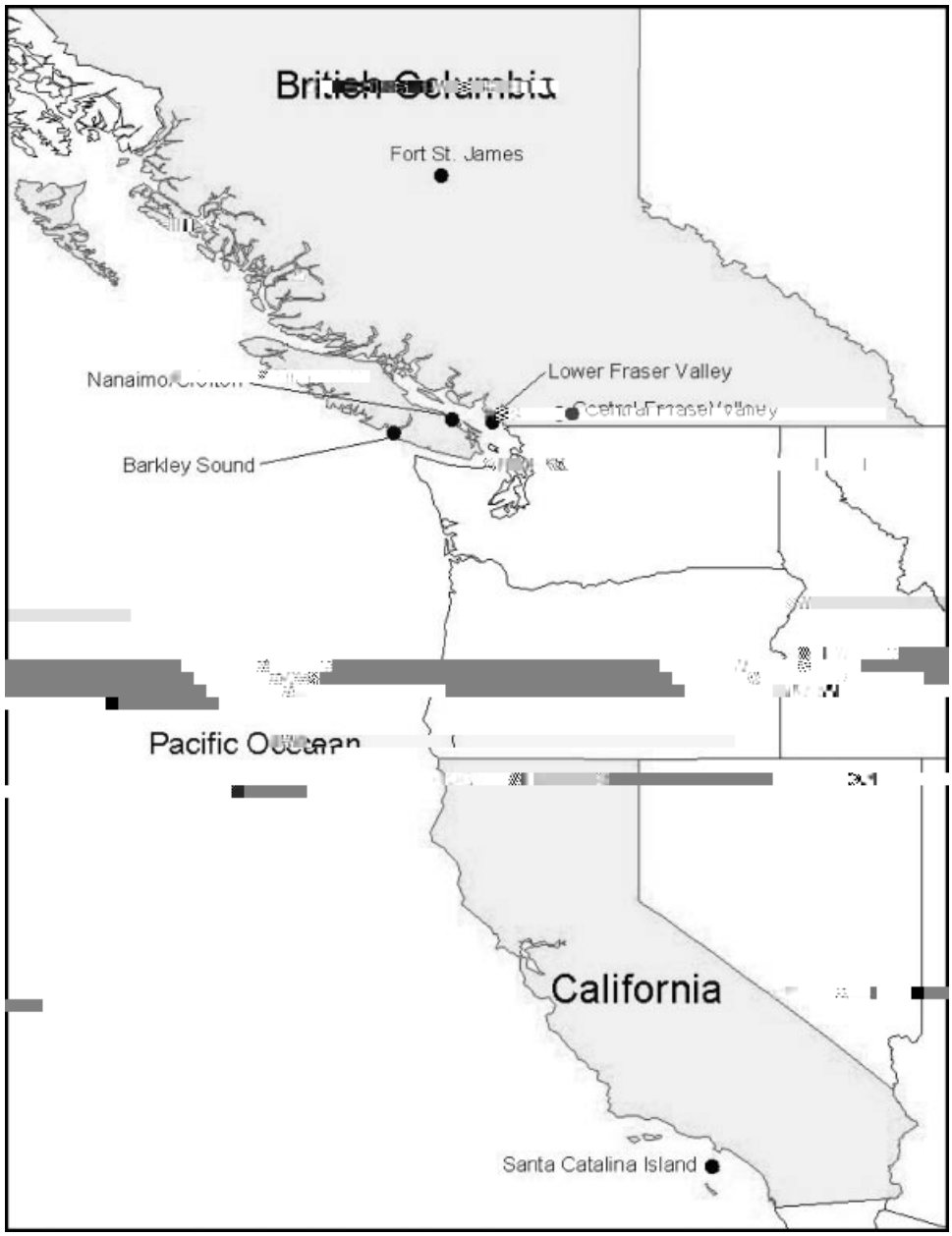

Fig. 1. Location of study sites.

hormone added and eluted from miniature Sephadex columns [39]. Columns were washed with sodium hydroxide and drained. Radiolabeled hormone was added to each column, followed by the addition of plasma. The columns were washed with phosphate buffer to remove plasma proteins, and labeled hormones were then counted. The index is calculated based on the formula

$$\text{FT4I or FT3I} = (\text{total hormone added} - \text{eluted [cpm]}) \times 100) \text{ total hormone added (cpm)} \times \text{dilution factor}$$

where FT4I and FT3I are relative measures of the binding potential of the plasma for the respective thyroid hormones, which are correlated with the percentage of free thyroid hormones [39]. A low index value is indicative of a high binding potential of the plasma for that particular thyroid hormone.

For retinol analysis, a 100-µl aliquot of plasma was extracted using retinyl acetate as the internal standard. The analysis was done using a Varian high-performance liquid chromatography system (9010-2332, 91000-2778) with an ultraviolet visible detector (9050-0664) set to 325 nm for the detection of retinoids. The calibration standard curve ranged from 0.8 to 17 ng per injection. A verification standard and a quality-assurance/quality-control plasma sample were included and analyzed with each set of samples for quality assurance. Retinol was also determined for the subset of 25 nestlings collected in 1998; methods have been described by Honour et al. [40]. Care was taken not to expose samples to light, which rapidly degrades retinol.

Statistical analyses

All analyses were performed using the statistical package R 2.4.1 (http://www.r-project.org/). All contaminants (p,p′-DDE,

PCBs, and PBDEs) were normally distributed after log transformation. The relationship between contaminants and colorimetrically determined lipid content was determined using linear regression models. Site differences were determined by analysis of variance, and to determine which sites were different a Student Newman Keuls test was performed. No correlation was found, so the contaminants were not normalized for lipids. Because the group of 25 nestlings collected in 1998 were analyzed using different laboratory techniques, a separate set of statistical analyses was conducted for that group.

The relationship between contaminants and thyroid hormones/retinol was also investigated using general linear models. Because all contaminants are intercorrelated (Table 1), and a causal relationship with one contaminant will often result in a spurious correlation with another, we used Akaike's information criterion (cAIC) to select which contaminants had the most support for explaining biomarker concentrations. The AIC method ranks models based on their overall statistical support. Lowest-AIC models are most supported, and AIC weights provide a measure of the relative support for each model. A two-process forward stepwise regression procedure was used because the sample size with legacy contaminant values was larger than the sample size with PBDE values. First, we selected the important parameters (contaminants) for T3, T4, and retinol models from the legacy contaminants: p,p′-DDE, ∑PCBs, CB153, CB99, CB118, CB138, CB180, and nestling age. Thyroxine was included as a possible parameter in the model for T3 and vice versa. Next, we included the significant parameters from that model into models that included ∑PBDEs, ∑OH-PBDEs, and ∑OH-PCBs. We report cAIC and AIC weights for different models.

RESULTS AND DISCUSSION

Contaminants and thyroid hormones

Results for all individual organochlorine (OC) pesticides from the 2003 study have been reported by Cesh et al. [33] and for PCB, PBDE, OH-PBDE, and OH-PCB congeners by McKinney et al. [35]. These results are available along with the individual hormone and retinol values in the Supplemental Data. In the present study, plasma samples from bald eagle nestlings showed that T3 correlated with retinol (2003 nestlings: $t_{32} = 2.48$ $p = 0.02$ $R^2 = 0.25$; 1998 nestlings: $t_{24} = 1.18$ $p = 0.25$ $R^2 = 0.10$; Fig. 2), whereas T4 was independent of both T3 (2003 nestlings: $t_{32} = 0.05$, $p = 0.95$, $R^2 = 0.00$; 1998 nestlings: $t_{24} = 1.04$, $p = 0.31$, $R^2 = 0.05$; adults: $t_{16} = 0.73$, $p = 0.44$, $R^2 = 0.02$) and retinol (2003 nestlings: $t_{32} = 0.72$, $p = 0.48$, $R^2 = 0.02$; 1998 nestlings: $t_{24} = 0.38$, $p = 0.71$, $R^2 = 0.01$). Because T4 was independent of T3, we did not examine the T4:T3 ratio. Models showed that T4 decreased with p,p′-DDE, CB153 and ∑PCBs or just CB153 (Table 2 and Fig. 3). Note that the AIC value for p,p′-DDE is so small that models with and without p,p′-DDE must be considered similarly supported (Table 2). Models also suggested that T3 decreased with p,p′-DDE and increased with ∑OH-PBDEs or decreased with both p,p′-DDE and ∑PCBs (Table 2 and Fig. 3). However, it should be recognized that the relationship between T3 and ∑OH-PBDEs is likely an artifact of the low concentrations of PBDEs at that remote, nonurbanized site combined with it being the only site without any marine input. Similarly, the relationship reported below of T3 and retinol is a similar artifact, given lower retinol concentrations in freshwater versus marine food chains [41]. In the 1998 samples, free levels of both T3 and T4 declined with ∑OH-PCBs and increased with CB-153 (Table 2 and Fig. 4). The present study showed that, in contrast p,p′-DDE and ∑PCBs, there were no significant correlations between ∑PBDEs and circulating thyroid hormones in nestling bald eagles, and only T3, but not T4, increased with ∑OH-PBDE (Fig. 3).

Previous studies have reported similar results for other bird species when investigating the correlations between p,p′-DDE and ∑PCBs with thyroid hormones (Tables 3 and 4). T4 declined with ∑PCBs in nine of 14 published studies, with four showing no effect. Thyroxine declined with p,p′-DDE for one of five studies, with four studies showing no effect (Tables 3 and 4). The only study that showed an opposite (increasing) trend for ∑PCBs showed complex temporal variation in the strength of the relationship based on time since the egg was laid [42]. Trends were less clear for T3. Five of nine studies showed no effect of ∑PCBs on T3 levels, and four showed a negative effect. All four studies showed no effect of p,p′-DDE on T3 levels (Tables 3 and 4).

To date, few studies have examined the effects of PBDEs

(Table 5) found an effect on T3 levels, and only one of two studies found a negative effect on T4 levels. An avian laboratory study found a significant negative trend between three PBDE congeners (PBDE-47, -99, and -100) but not $\sum$PBDEs [44], although there were no significant differ-

system, which increases the risk of a prolonged disruption of this system and associated physiological functions, including developmental disturbances [1,2,21].

For birds, research has focused on the effects of contaminants on the transport proteins. The thyroid hormone transport system consists of a complex of two proteins: TTR, which contains two binding pockets for thyroid hormones, and a retinol binding protein (RBP), which contains a binding site for the vitamin A analog retinol [1,21]. Some of the contaminants examined in this study have molecular structural similarities to T4, allowing those chemicals to mimic T4 and bind to TTR [23,49,53]. Those contaminants (and especially their hydroxylated metabolites) also appear to have a higher binding affinity than T4, which is out-competed and displaced from its binding sites on TTR [1,21,53].

A recent study examined the capacity of PCB and PBDE

levels are decreased with increased or normal T3 (or reversed T3), and TSH levels become unregulated by pituitary and thyroid gland interactions [2].

It is beyond the scope of the present paper and the insights afforded by our data to consider all alternate mechanisms in detail. However, a recent in vitro study showed that OH-PBDEs act as thyroid hormone-like agents, with meta- or para-

shown a decrease in retinol levels after exposing rats and mice to PCBs and PBDEs [4]. Thus, although PCBs and OH-BDEs likely affect retinol levels, the direction of the relationship is difficult to predict, perhaps calling into question the utility of the simple measurement of circulating retinol as an effective

43. Zhou T, Ross DG, DeVito M, Crofton KM. 2001. Effects of short term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones