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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, perhaps because adult birds have time to adjust to contaminant levels. Measurement of circulating thyroid hormones appears to be a more useful biomarker than retinols, given the more consistent response of T4 to PCBs here and reported in the literature. We conclude that current environmental exposures to PCBs in British Columbia and in southern California are associated with significant decreases in T4, suggesting a potential negative effect on the endocrine system of nestling bald eagles. Environ. Toxicol. Chem. 2010;29:1301–1310. © 2010 SETAC

Keywords—Contaminants Thyroid hormone Retinol Bald eagle PCBs

INTRODUCTION

Contamination of the global environment with polyhalogenated aromatic hydrocarbons (PHAHs), many of which are considered to be endocrine disruptors, has been well documented [1–6]. Among the most persistent and commonly detected compounds of this group are dichlorodiphenyltrichlorethane (*p,p′*-DDT), its metabolite 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p,p′*-DDE), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzo furans (PCDFs). During the latter half of the twentieth century, large quantities of such legacy PHAHs were released to the global environment by various industrial and agricultural activities, which have now been largely curtailed by regulations and international agreements (http://www.pops.int/and http://www.unece.org/env/lrtap/pops_h1.htm). A group of PHAH chemicals of more recent concern is the polybrominated diphenyl ethers (PBDEs) [7–9]. Polybrominated diphenyl ethers are flame retardants with physical–chemical properties and environmental persistence similar to those of PCBs and also appear to be dispersing similarly throughout the environment [9]. Polybrominated diphenyl ethers are persistent and bioaccumulative, although there is some evidence that they do not biomagnify with trophic level in homeothermic wildlife to the same degree as PCBs [10,11]. Trends in PBDEs in wildlife samples appear to have tracked usage patterns in various jurisdictions, increasing until the mid-1980s and then decreasing in Baltic murres (*Uria algae*) [12] and increasing exponentially in North American and arctic wildlife into the early 2000s [13–16]. However, studies on Great Lakes herring gull (*Larus argentatus*) eggs have recently shown a considerable slowing in the temporal rate of PBDE levels, which increased between 1991 and 2006 [17]. In the last several years, no further increases in penta- and octa-BDE derived congeners were noted in the gull eggs, and the concentrations may actually be decreasing [17]. Given the overall indications of persistence and tendency to bioaccumulate, and with recent evidence of their toxicity at environmental concentrations, there is some concern that PBDEs may have consequences for survival and/or reproduction of wildlife populations.

From both laboratory and field studies, PHAHs have a broad range of effects in wild birds [18]. In particular, many of these compounds have been shown to compromise thyroid function, specifically altering normal synthesis, secretion, transport,

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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 landfills 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 then discuss our data in the context of a literature review of results from previously published studies on PHAHs and the thyroid system in birds.

MATERIALS AND METHODS

In 2003, blood samples were obtained from five sites in British Columbia and one site in California as described by Cesh et al. [33] (Fig. 1). Plasma samples from 34 bald eagle nestlings were analyzed, and wet weight concentrations for contaminants are presented in the Supplemental Data. Because of low sample volume, samples were pooled for the southern California PBDE, OH-PBDE, and OH-PCB data. In 1998, plasma samples for an additional 25 nestlings were collected using methods comparable to those for the nestlings collected in 2003 [33]. In total 17 adult bald eagles were trapped, and a blood sample collected according to methods described elsewhere [33] and at locations similar to those for the nestlings sampled on the west and east coasts of Vancouver Island. Samples were analyzed for thyroid hormones, retinols, *p,p′*-DDE, PCBs, and OH-PCBs but not PBDEs.

Contaminant analyses

Sample analysis for *p,p′*-DDE and PCBs followed standard procedures [11,34]. Samples collected in 2003 were also analyzed for PBDEs (total PBDEs: BDEs 47, 99, 100, 138, 153, 183, and 209), pentabromobiphenyl BB101, and total hexbromocyclododecane (total HBCD). Procedures used for the extraction and determination of PBDEs in plasma have been described elsewhere [35]. Mean recovery (±1 SE) of the internal standards was on average 90 ± 7% for BDE30. Concentrations were recovery corrected, because an internal standard method of quantification was used to reduce heterogeneity within and between analyte classes. Method blank samples were included with each batch of five samples to monitor interferences and coeluting contamination. No substantial background contamination was encountered for any BDE or halogenated phenolic analytes. The analytical precision of quantitative determinations was tested by repeated injections of standard compounds into quality-control samples, and, when sample amount permitted, duplicate analyses of selected bald eagle samples was performed. Duplicate samples demonstrated on average 10% variation of analyte concentrations. The method limit of quantification (MLOQ) for individual analytes was based on a minimum of 10 times the noise level (S/N). The MLOQ in bald eagles ranged between 0.001 and 0.01 ng/g wet weight.

Contaminant levels in the subset of 25 nestlings and 17 adults in 1998 were measured as described elsewhere [36–38]. Plasma samples were spiked with internal standards prior to extraction. Adult plasma samples (2–5 g) were spiked with $^{13}C_{12}$-PCB mix (10 μl at 2 ng/μl) and $^{13}C_{12}$-HO-PCB mix (20 μl at 100 pg/μl). Nestling plasma samples (8–12 g) were spiked with $^{13}C_{12}$-PCB mix (10 μl) and $^{13}C_{12}$-HO-PCB mix (15 μl). Gas chromatography–mass spectrometry (GC-MS) analyses were performed on a Hewlett-Packard 5988A mass spectrometer coupled with a Hewlett-Packard 5890A Series II gas chromatograph equipped with a Hewlett-Packard 7673A automatic sampler. Samples were analyzed in selected ion-monitoring mode by GC-MS using electron capture negative ionization. Authentic standards were used to generate relative response factors (RRFs). The external standard method was used to quantify all identified and unidentified OH-PCBs. Unidentified OH-PCBs were characterized by chlorination pattern to select an appropriate RRF for quantification.

Thyroid hormone and retinol analysis

Different methods were used in 1998 and 2003, so analyses were completed separately for the two groups. In 2003, thyroid hormones T4 (total T4) and T3 (total T3) were analyzed using coat-a-count canine T4 and T3 kits (Diagnostic Products). These solid-phase radioimmunoassays use tubes coated with monoclonal T4 or T3 antibodies. After an adequate incubation period, bound and free fractions are separated and the radioactivity quantified. Sample results are interpolated from a standard curve generated by counting samples containing known quantities of unlabeled T4 or T3. For quality control, commercial samples (canine serum) of T4 or T3 were analyzed with each series of samples, and all were within the acceptable range. Samples were also analyzed in duplicate. As a result of the demands on the amount of plasma for the comprehensive chemistry determinations, there was no plasma remaining to validate specifically the thyroid assays for the bald eagle. However, we previously validated the assay for reproducibility, recovery, parallelism, and intra-assay precision for six other species (Trudeau and Maisonneuve, unpublished data), including three birds of prey, osprey (*Pandion haliaetus*), American kestrel (*Falco sparverius*), and peregrine falcon (*Falco peregrinus*). The assay performed consistently for those six species, so we have confidence in its application to the bald eagle.

For the subset of 25 nestlings and 17 adults collected in 1998, total T4 and T3 (TT4 and TT3) were measured by radioimmunoassay [39]. Free T4 and T3 indices (FT4I and FT3I) were determined by measuring (counts per minute) radiolabeled

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 ΔcAIC 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 ΔAIC 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 to *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).

Table 1. Correlation coefficients for different contaminants[a]

	p,p′-DDE	∑PCB	∑PBDE	∑OH-PBDE
∑PCB	*0.51*	—	—	—
∑PBDE	*0.44*	*0.47*	—	—
∑OH-PBDE	0.14	*0.26*	*0.45*	—
∑OH-PCB	*0.48*	*0.31*	*0.59*	*0.25*

[a] All regressions were made on log-transformed values. Values significant at $p < 0.01$ are in italics. *p,p′*-DDE = 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethylene; PCB = polychlorinated biphenyl; PBDE = polybrominated diphenyl ethers; OH-PBDE = hydroxylated polybrominated diphenyl ethers; OH-PCB = hydroxylated polychlorinated biphenyls.

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 in wild birds; most studies have been performed in the laboratory. Although lower T4, and to a lesser degree T3, levels have been reported in mammals exposed to PBDEs [2,4,43], neither of the two presented avian studies on PBDEs

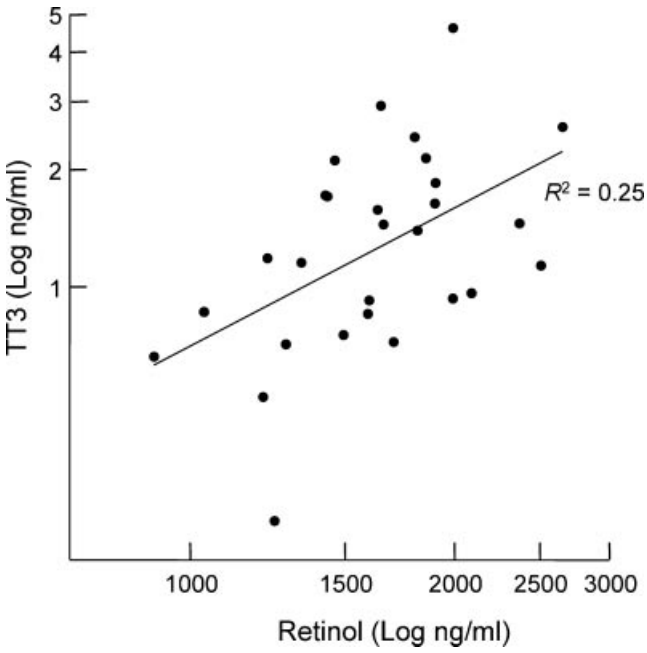

Fig. 2. Total triiodothyronine (TT3) levels increase with retinol levels for nestling bald eagles from British Columbia, Canada, and California, USA, collected in 2003.

Table 2. cAIC values and cAIC weights for models with cAIC > 2.0 predicting the different response variables for bald eagle nestlings from western North America[a]

Response	Predictors	ΔcAIC	Weights
Retinol (2003)	∑OH-PBDE + CB138 + CB180	0.00	0.23
	CB138 + CB180	0.05	0.22
	CB180	1.37	0.11
Retinol (1998)	∑OH-PCB	0.00	0.50
	∑OH-PCB + *p,p′*-DDE	1.07	0.29
Total T3 (2003)	∑OH-PBDE	0.00	0.21
	∑OH-PBDE + *p,p′*-DDE	0.05	0.21
	∑OH-PBDE + *p,p′*-DDE + CB-118	0.49	0.17
Total T3 (1998)	∑PCB	0.00	0.50
	p,p′-DDE	0.59	0.37
	p,p′-DDE + ∑PCB	1.26	0.37
Free T3 (1998)	∑OH-PCB + CB153	0.00	0.50
	∑OH-PCB + CB153 + *p,p′*-DDE	1.75	0.21
Total T4 (2003)	CB153	0.00	0.21
	CB153 + ∑PCB	0.96	0.13
	CB153 + ∑PCB + ∑OH-PCB	1.19	1.12
	CB153 + ∑PCB + *p,p′*-DDE	1.30	0.11
	∑PCB	1.66	0.09
Total T4 (1998)	CB153	0.00	0.32
Free T4 (1998)	∑OH-PCB + CB153	0.00	0.55
	∑OH-PCB + CB153 + *p,p′*-DDE	0.82	0.36

[a] Values and weights for 25 bald eagle nestlings sampled in 1998 are also shown for each response variable; samples collected in 1998 were also analyzed for free thyroid hormone levels. No model was better than the nul model (no effect) for adult bald eagles. cAIC = Akaike's Information Criterion; OH-PBDE = hydroxylated polybrominated diphenyl ether; OH-PCB = hydroxylated polychlorinated biphenyl; PCB = polychlorinated biphenyl; *p,p′*-DDE = 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; T3 = triiodothyronine; T4 = thyroxine.

(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 (PBDEs −47, −99, and −100) but not ∑PBDEs [44], although there were no significant differences in T4 between dosed and control groups. In that study, whole-body concentrations of ∑PBDEs were 86 ng/g in kestrel nestlings, which is difficult to compare with our study, in which plasma concentrations were 5 ng/g (mean) or 30 ng/g (maximum). Similarly, the other two studies on ∑OH-PCBs found no effect on T3 or T4 levels (Table 5). There has been only one other study on ∑OH-PBDEs, and that study found no effect on T3 or T4 levels (Table 5). We conclude that PBDEs did not influence thyroid hormones in eagles at environmentally relevant concentrations but that ∑OH-PBDEs might have. However, research on thyroid hormone disruption of populations of wild birds highly exposed to PBDEs, such as urban peregrine falcons (*Falco peregrinus*), is indicated [45].

Thyroid hormones play different roles in developing and adult animals. In the developing animal, thyroid hormones are associated with growth and development; in the adult animal, thyroid hormones are associated primarily with metabolism, especially basal metabolic rate. For that reason, thyroid hormone levels vary with age and tend to be lower in older relative to younger animals. Circulating thyroid hormone levels were lower in adult (T3: 0.22 ± 0.06 nmol/L; T4: 20.3 ± 4.15 nmol/L) compared with nestling (T3: 0.70 ± 0.10 nmol/L; T4: 29.1 ± 3.0 nmol/L) eagles in our study. The different role of thyroid hormones during development may explain why we found no relationship between contaminants and thyroid levels in adult eagles, despite much higher contaminant levels in adults [11]. Similarly, whereas three of five published studies of PCBs and T3 in developing (egg/young) birds showed a negative relationship, only one of four studies in adult long-lived birds (raptors and seabirds) showed a negative relationship. Perhaps older birds have time for their thyroid axis to adapt or acclimate to higher contaminant levels, or perhaps the lower rate of metabolic and growth processes, as shown by lower thyroid hormone levels, means that adult birds can adjust through other mechanisms when the thyroid axis is affected by a foreign pharmacologic agent.

Many natural and experimental factors can confound the apparent relationships among T3, T4, and contaminants and may explain some of the no effects or opposing effects shown in Tables 3, 4, and 5. Confounding influences include gender [46]; correlative relationships with other compounds that occur in higher concentrations and also impact thyroid hormones [47]; and timing, insofar as hormones change over short time scales, and even small differences in sampling age could alter perceived relationships [42,48]. Methodological issues include whether free versus bound hormones are measured (Table 2; [50]) and whether plasma or thyroid gland T4 content is measured, with the latter suggested as a better measure of endocrine disruption [51]. Furthermore, the T4-binding site on transthyretin (TTR) in birds has a lower affinity for T4 than that in mammals, which may also play a role in the fewer negative correlations for T4 levels in birds compared with mammals [21]. Thus, PCBs and *p,p′*-DDE decrease T4 in birds, at least when levels are high enough and confounding variables are unimportant. PCBs and *p,p′*-DDE may also reduce T3 levels, but studies to date are inconclusive. PCBs continue to exert an effect on the thyroid hormone system in some wild bird populations, evident from statistical correlations with circulating T4 levels, but future studies should attempt to examine some of the above-mentioned methodological issues.

Contaminants can affect production and functioning of thyroid hormones at a number of stages [52], including synthesis of thyroid hormones, thyroid hormone transport binding proteins [1,2,21], cellular uptake mechanisms, the thyroid receptor, thyroid conversion by iodothyronine deiodinases, and metabolism of thyroid hormones in the liver. Contaminants may interfere at one or all levels of the thyroid hormone

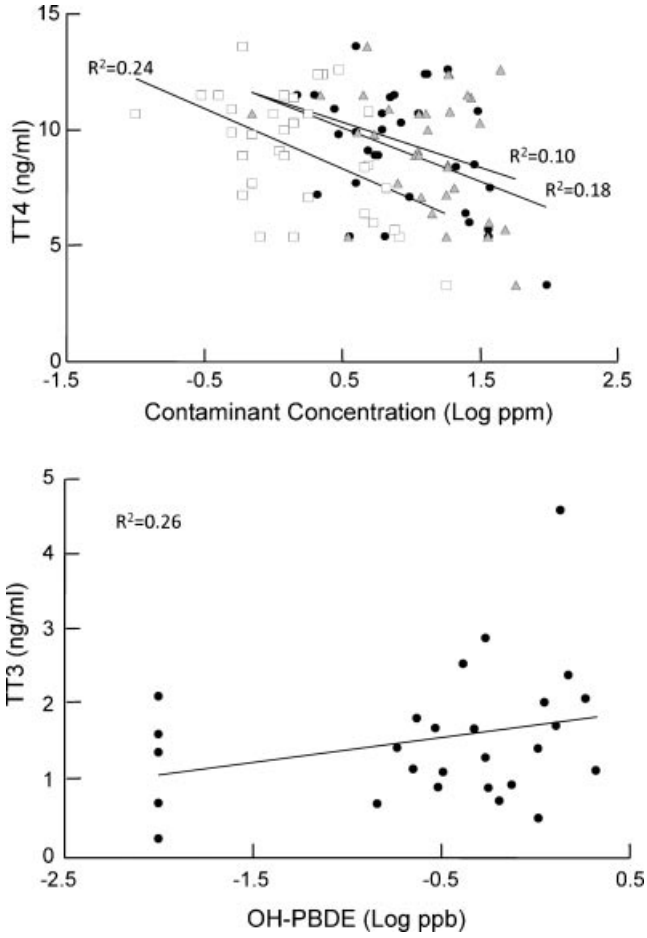

Fig. 3. Total thyroxine (TT4; top) decreased and total triiodothyronine (TT3; bottom) increased with contaminant concentrations for nestling bald eagles collected in 2003. Relationships are shown for contaminants selected in lowest Akaike's information criterion models. In upper graph, squares represent CB153, circles represent sum polychlorinated biphenyls (∑PCB), and triangles represent 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p,p′*-DDE). Dashed line represents linear CB153, black solid line represents linear ∑PCB, and gray solid line represents linear *p,p′*-DDE. OH-PBDE = hydroxylated polybrominated diphenyl ether.

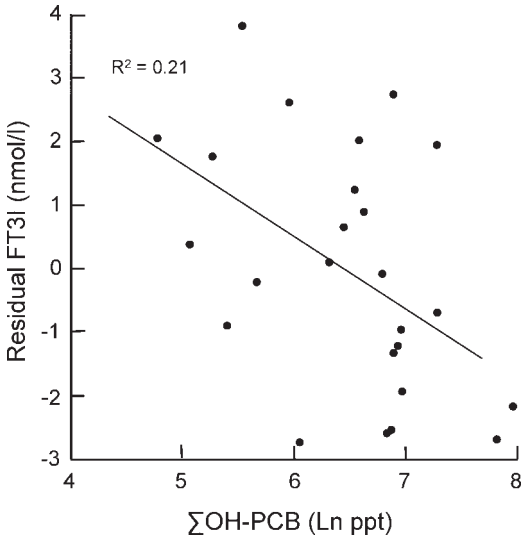

Fig. 4. Residual free T3 index (FT3I; accounting for polychlorinated biphenyls [PCB] 153) decreased with sum hydroxylated polychlorinated biphenyls (∑OH-PCBs) for nestling bald eagles collected in 1998 from British Columbia, Canada. T3 = triiodothyronine.

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 congeners and their hydroxyl (OH)- and methoxyl (MeO)-containing analogs (i.e., 4-OH-CB187, 6-OH-BDE47, 4-OH-BDE49, 4-MeO-CB187, and 6-MeO-BDE47) to bind competitively with glaucous gull TTR protein. The OH-substituted analogs 4′-OH-BDE49 and 6-OH-BDE47 were more potent at binding to the TTR than either T3 or T4, whereas non-OH-PBDE molecules were less potent [26]. Both 4′-OH-BDE49 and 6-OH-BDE47 were detected in the plasma of Svalbard glaucous gulls [54]. Furthermore, in general, OH-PBDEs were more effective competitive ligands for either T3 or T4 than, for example, OH-PCBs, because of the combination of the more thyroid hormone-like brominated diphenyl ether backbone relative to the chlorinated biphenyl backbone [26]. However, the most effective congener (4′-OH-BDE49) was not detected in our samples. Thus, although our results suggest that PCBs and *p,p′*-DDE are most directly related to the inhibition of T4, they also suggest that OH-PBDEs may act to increase free T3, as suggested by the above-described studies. In the 1998 data set, OH-PCBs were strongly associated with declines in free thyroid levels. The lesser role of OH-PCBs compared with PCBs in the regulation of thyroid hormones in the 2003 data set, despite the biomolecular studies to the contrary, may reflect the much lower concentrations of OH-PCBs compared with PCBs. Indeed, our average ∑PCB concentration was about 15,000 ppb, whereas our average ∑OH-PCB concentration was about 0.7 ppb, with the relative concentrations of individual congeners being somewhat similar. The 1998 study, which did find a relationship between free thyroid hormone levels and ∑OH-PCB levels, had ∑OH-PCB levels that were about twice as high (e.g., mean 3-OH-46 = 0.27 ppm instead of 0.13 ppm). Thus, although in vitro studies of seabird tissue demonstrate that OH-PCB congeners out-compete T4 at tenfold lower concentrations and T3 at 100-fold lower concentrations (see Fig. 5 of Ucan-Marin et al. [26]), PCBs were over 20,000 times more abundant, so no relationship was observed in 2003. Furthermore, one reason that relationships are consistently found between PCBs and T4, rather than T3, may be because OH-PCBs affect TTR–T3 binding at much lower concentrations than TTR–T4 binding [26].

The effect of the competitive inhibition of T4 over moderate time scales is to reduce the amount of T4 reaching the target tissues, with the unbound fraction of T4 thus being more readily excreted. Also, there may be a temporary increase in the level of free T4 in the plasma, which may increase enzymatic degradation of T4 [2]. As a result of increased degradation of T4, its

Table 3. Summary of studies investigating the effect of polychlorinated biphenyls (PCBs) on thyroid hormones and retinol including type of study and stage of life[a]

Species	Type	Stage	T3	T4	Retinol	Source
Common eider (*Somateria mollissima*)	Field	Young			N	58
Chicken (*Gallus gallus domesticus*)	Lab	Egg	N	+		42
Chicken (*Gallus gallus domesticus*)	Lab	Egg		–[b]		48
Chicken (*Gallus gallus domesticus*)	Lab	Egg	–	–		59,60
Japanese quail (*Coturnix coturnix japonica*)	Lab	Young			–	61
Japanese quail (*Coturnix coturnix japonica*)	Lab	Adult		N		62
Great cormorant (*Phalacrocorax carbo*)	Field	Young	N	–[c]		50
European shag (*Phalacrocorax aristotelis*)	Field	Young			–	63
Great blue heron (*Ardea herodias*)	Field	Egg			–	64
Great blue heron (*Ardea herodias*)	Field	Young	–	N	–	64
Osprey (*Pandion haliaetus*)	Field	Young			+	65
Bald eagle (*Haliaeetus leucocephalus*)	Field	Young	N	–	+	This study
Bald eagle (*Haliaeetus leucocephalus*)	Field	Adult	N	N		This study
American kestrel (*Falco sparverius*)	Lab	Young	–	–		66
American kestrel (*Falco sparverius*)	Lab	F1	–	–		66
Common tern (*Sterna hirundo*)	Field	Egg			+	6
Common tern (*Sterna hirundo*)	Lab	Young		–		67
Caspian tern (*Sterna caspia*)	Field	Young			–	68
Black-legged kittiwake (*Rissa tridactyla*)	Field	Young			N	69
Herring gull (*Larus argentatus*)	Field	Young		–		70
Herring gull (*Larus argentatus*)	Field	Young			–	68
Herring gull (*Larus argentatus*)	Field	Adult	N	–		71
Glaucous gull (*Larus hyperboreus*)	Field	Adult	N	N		72
Glaucous gull (*Larus hyperboreus*)	Field	Adult	N	–		46
Thick-billed murre (*Uria lomvia*)	Field	Young			N	58
Black guillemot (*Cepphus grille*)	Field	Young			–	73
Cave/cliff swallows (*Petrochelidon fulva/Petrochelidon pyrrhonota*)	Field	Adult		N		74

[a] N implies no effect, + implies the compound is higher with increasing PCBs and – implies the compound is lower with increasing PCBs. T3 = triiodothyronine; T4 = thyroxine; PCB = polychlorinated biphenyl.
[b] A negative effect in relation to Aroclor 1242 and 1254.
[c] A negative effect on free T4 only.

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 a *meta-* or *para-*OH group and adjacent dibromo substitution necessary for OH-PBDEs to function as thyroid hormone receptor agonists [25]. Another biomolecular study reported that nondioxin-like PCBs inhibit the binding of T3 to TTR and suppress TTR action by dissociating thyroid hormone receptors (TR) from the thyroid hormone response element through interactions with the TR–DNA binding domain [55].

Contaminants and retinol levels

In plasma samples from nestlings, retinol increased with ∑OH-PBDE, CB138, and CB180 in 2003 and OH-PCBs in 1998 (Table 2 and Fig. 5). No significant correlations were found between *p,p′*-DDE and PBDEs with retinol (Table 2). Across a variety of other avian studies, three of 12 found no effect of PCBs, two found positive relationships between retinol and PCBs, and seven found negative relationships (Table 3). All other published studies found no effect for *p,p′*-DDE (Table 4). No previous study had examined relationships between OH-PCBs and OH-PBDEs and retinol, but, of five studies examining ∑PBDEs and retinol, four found no effect and one found a negative effect (Table 5). However, laboratory studies have

Table 4. Summary of studies investigating the effect of *p,p′*-DDE on thyroid hormones and retinol including type of study and stage of life[a]

Species	Type	Stage	T3	T4	Retinol	Source
Common eider (*Somateria mollissima*)	Field	Young			N	58
European shag (*Phalacrocorax aristotelis*)	Field	Young			N	63
Bald eagle (*Haliaeetus leucocephalus*)	Field	Young	–	–	N	This study
Bald eagle (*Haliaeetus leucocephalus*)	Field	Adult	N	N		This study
Glaucous gull (*Larus hyperboreus*)	Field	Adult	N	N		72
Glaucous gull (*Larus hyperboreus*)	Field	Adult	N	N		46
Thick-billed murre (*Uria lomvia*)	Field	Young			N	58
Cave/cliff swallows (*Petrochelidon fulva/Petrochelidon pyrrhonota*)	Field	Adult		–		74
Tree swallow (*Tachycineta bicolor*)	Field	Young	N	N[b]		47
Eastern bluebird (*Sialia sialis*)	Field	Young	N	N		47

[a] N implies no effect, + implies the compound is higher with increasing *p,p′*-DDE, and – implies the compound is lower with increasing *p,p′*-DDE. *p,p′*-DDE = 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; T3 = triiodothyronine; T4 = thyroxin.
[b] Although a positive trend for DDE was observed, in a multivariate model only other pesticides had support.

Table 5. Summary of studies investigating the effect of PBDEs, OH-BDEs, and OH-PCBs on thyroid hormones and retinol including type of study and stage of life[a]

Species	Chemical	Type	Stage	T3	T4	Retinol	Source
Mallard (*Anas platyrhynchos*)	PBDE	Lab	Egg			N	75
Common eider (*Somateria mollissima*)	PBDE	Field	Young			N	58
Bald eagle (*Haliaeetus leucocephalus*)	PBDE	Field	Young	N	N	N	This study
American kestrel (*Falco sparverius*)	PBDE	Lab	Egg	N	−	−	44
Black-legged kittiwake (*Rissa tridactyla*)	PBDE	Field	Young			N	63
Glaucous gull (*Larus hyperboreus*)	PBDE	Field	Adult	N	N		72
Thick-billed murre (*Uria lomvia*)	PBDE	Field	Young			N	58
Japanese quail (*Coturnix coturnix japonica*)	OH-PCB	Lab	Adult	N	N		76
Bald eagle (*Haliaeetus leucocephalus*)	OH-PCB	Field	Young	−	N	−	This study
Bald eagle (*Haliaeetus leucocephalus*)	OH-PCB	Field	Adult	N	N		This study
Glaucous gull (*Larus hyperboreus*)	OH-PCB	Field	Adult	N	N		72
Bald eagle (*Haliaeetus leucocephalus*)	OH-PBDE	Field	Young	+	N	+	This study
Glaucous gull (*Larus hyperboreus*)	OH-PBDE	Field	Adult	N	N		72

[a] N implies no effect, + implies the compound is higher with increasing PBDEs or metabolites, and − implies the compound is lower with increasing PBDEs or metabolites. PBDE = polybrominated diphenyl ether; OH-BDE = hydroxylated brominated diphenyl ether; OH-PCB = hydroxylated polychlorinated biphenyl; T3 = triiodothyronine; T4 = thyroxin.

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 biomarker of PHAHs.

Retinol is also affected by contaminants binding to TTR [53,56,57]. Normally, retinol binds to RBP and then as a complex binds to TTR, which is transported to the target tissues [2]. Contaminants may interfere with this process by binding to TTR, causing a conformational change that prevents the RBP from binding to TTR, thereby preventing effective transport of retinol to its target tissues. Consequently, retinol is left unbound, which increases its chances of being excreted [2]. However, many of the confounding factors listed for thyroid hormones are also important for studies of retinol, and, although many studies show a relationship between retinol and contaminants, the direction of the relationship is variable (Tables 3–5).

Our study of bald eagles may support the idea that contaminants interfere with the thyroid hormone system through interactions with plasma transport processes for both thyroid hormones and retinol (i.e., TTR and RBP). Retinol and the thyroid hormones are both transported via the same transport protein (TTR) [23,53], so one would expect similar effects given that contaminants are affecting the transport protein. In confirmation, we saw similar relationships for contaminants, T3, and retinol and tight correlation between T3 and retinol (Tables 1 and 2). However, T4 was independent of T3 and retinol and was the only variable to show a strong relationship with ∑PCB. Thus, although T3 and retinol may be impacted by contaminants at the same step of the transport mechanism, it appears that T4 may be impacted at a different step. We suggest that thyroid hormones are better biomarkers for contamination than retinol, in that thyroid hormones consistently decrease with contamination, whereas retinol shows variation in both the existence and the direction of responses.

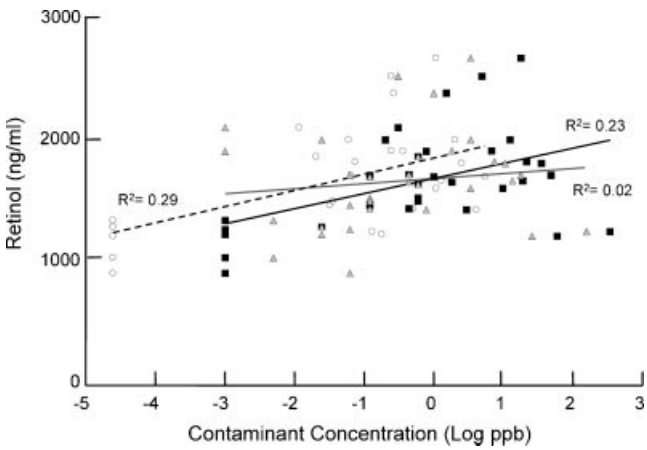

Fig. 5. Retinol increased with contaminant concentrations in the plasma of nestling bald eagles in British Columbia, Canada, collected in 2003. Circles represent sum hydroxylated polybrominated diphenyl ether (∑OH-PBDE), squares represent CB138, and triangles represent CB180. Black solid line represents linear CB138, dashed line represents linear ∑OH-PBDE, and gray solid line represents CB180.

SUMMARY

The present study showed a decrease in T4 and T3 in relation to *p,p′*-DDE and, consistently with many other studies, a decrease in T4 in relation to PCBs in bald eagle nestlings. These results suggest that PCBs in particular do have some continuing negative effect on the endocrine system of nestling bald eagles. In contrast, PBDEs did not show a correlation with either thyroid hormone (T3 or T4) or retinol, although their hydroxylated analogues or metabolites may alter the abundance of T3 and retinol. Currently, however, bald eagle populations, including those in the Georgia Basin, are generally stable or increasing, which is evidence that any such effects, alone or with other stressors, are not significantly affecting survival or reproduction. Nevertheless, our data and data from the literature indicate that local populations of avian top predators inhabiting areas of continuing high exposure to PCBs, and possibly PBDEs, may be subject to disruption of physiologically vital thyroid and retinol processes. Thus, further research with both laboratory models and wild birds would strengthen the database on the effects of environmental contaminants (especially the role of PBDEs and hydroxylated PBDEs and PCBs) on thyroid hormones and retinol.

SUPPLEMENTAL DATA

Table S1. Thyroid hormone, vitamin A, and organochlorine concentrations in nestling bald eagle plasma (2003). (34 KB XLS)

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