

Relationships between PCBs and Thyroid Hormones and Retinol in Female and Male Polar Bears

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We studied the relationships between polychlorinated biphenyls (PCBs) and thyroid hormones (THs) and retinol within two groups of female polar bears (*Ursus maritimus*), females with cubs of the year (FWCOY) and females without cubs of the year (FWOCYO), and within a group of males. Concentrations of five of the six quantified PCB congeners, i.e., PCB-99, PCB-153, PCB-156, PCB-180, PCB-194 (Σ PCB₅), correlated with each other, whereas the concentrations of PCB-118 did not correlate with the other congeners. Σ PCB₅ and PCB-118 did not differ between the three different groups of polar bears, and the plasma levels ranged from 16.7 to 203.2 ng/g wet weight (ww) for Σ PCB₅ and from 0.09 to 0.93 ng/g ww for PCB-118. PCBs did not affect the retinol status in any of the three groups. In FWCOY, we found negative correlations between Σ PCB₅ and the three TH variables free thyroxine (FT₄) ($r^2 = 0.35$), free triiodothyronine (FT₃) ($r^2 = 0.30$), and the total T₄:total T₃ ratio (TT₄:TT₃) ($r^2 = 0.92$). In FWOCYO, Σ PCB₅ was negatively correlated to TT₄ ($r^2 = 0.14$) and positively correlated to TT₃:FT₃ ($r^2 = 0.31$), whereas PCB-118 was positively correlated to FT₃ ($r^2 = 0.21$) and negatively correlated to TT₃:FT₃ ($r^2 = 0.26$). In males, Σ PCB₅ was negatively correlated to FT₃ ($r^2 = 0.56$) and positively correlated to FT₄:FT₃ ($r^2 = 0.78$), whereas PCB-118 was negatively correlated to FT₄:FT₃ ($r^2 = 0.53$). Thus, PCBs affected five TH variables in the female polar bears (TT₄, FT₄, FT₃, TT₃:FT₃, TT₄:TT₃), but PCBs affected only two TH variables in males (FT₃, FT₄:FT₃). Female polar bears could be more susceptible to TH-related effects of PCBs than are males. PCBs also affected T₃ to a larger degree than T₄. **Key words:** Arctic, endocrine disruption, hypothyroid, pollution, Svalbard, *Ursus maritimus*. *Environ Health Perspect* 112:826–833 (2004). doi:10.1289/ehp.6809 available via <http://dx.doi.org/> [Online 4 February 2004]

Organochlorines (OCs) are industrial chemicals, by-products in the chemical industry, or pesticides, and may exert toxic effects on organisms. Examples of OCs are polychlorinated biphenyls (PCBs), dioxins, chlorobenzenes, DDT, and lindanes. Virtually none of the OCs occur naturally (de March et al. 1998). The physical and chemical properties of OCs, such as their semivolatile nature, can result in repeated redistillation, which allows them to disperse widely via atmospheric transport (Barrie et al. 1992). Because volatile and persistent substances eventually condense in cold areas, OCs are prone to be deposited and accumulated in polar regions (Wania and Mackay 1993).

The polar bear (*Ursus maritimus*) is the ultimate apex predator in the Arctic food web, preying mainly on marine mammals such as ringed seals (*Phoca hispida*), bearded seals (*Eringnathus barbatus*), and harp seals (*Phoca groenlandicus*) (Derocher et al. 2002). Because many OCs are lipophilic and resistant to physical and biochemical degradation, they tend to accumulate in fatty tissues and are biomagnified in the food chain (de March et al. 1998; Fisk et al. 2001). Different OCs can vary in resistance to biochemical degradation

and toxic properties. Polar bears have an effective cytochrome P450 system and can therefore effectively metabolize many of the OCs they ingest from their prey, such as most of the PCB congeners and DDT and its metabolites (Bernhoft et al. 1997; Letcher et al. 1998). However, because polar bears consume large amounts of seal blubber, the most persistent PCB congeners can reach very high concentrations. The six congeners PCB-99, PCB-118, PCB-153, PCB-156, PCB-180, and PCB-194 are the most abundant and/or toxicologically important in polar bears (Bernhoft et al. 1997; Norstrom et al. 1998; Skaare et al. 2000). In polar bears from Svalbard, Norway, concentrations of the most persistent PCB congeners increased by up to 10 times from 1967 to 1994/1995 (Derocher et al. 2003), after which the concentrations seem to have stabilized (Henriksen et al. 2001).

The PCB burdens in polar bears in the Svalbard area are higher than in most of the North American ecosystems (Bernhoft et al. 1997; Letcher et al. 1995; Norstrom et al. 1998), but still somewhat lower than around Franz Josef Land and Novaja Zemlja (Andersen et al. 2001; Lie et al. 2003) in the Russian Arctic. The spatial differences in the

PCB concentrations in polar bears are probably related to the spreading of the compounds via prevailing ocean currents and wind systems, and biological factors such as spatial behavior and favored prey species of the bears (Olsen et al. 2003).

During the last decade there has been increasing concern about the endocrine-disruptive effects of many OCs in humans as well as in wildlife species (Brouwer et al. 1999; Rolland 2000; Vos et al. 2000). The thyroid system is one of the endocrine systems affected by PCBs (Brouwer et al. 1998). Alterations in thyroid hormone (TH) level or responsiveness to THs have significant behavioral, neurologic, and neuropsychologic consequences throughout the life cycle (Sher et al. 1998). THs also stimulate the uptake of oxygen in most metabolically active tissues, and TH regulation is essential for maintaining normal body temperature (McNabb 1992). Several reports have documented dose relationships between OCs and several cognitive and neurologic factors (Sher et al. 1998). Zoeller and colleagues have suggested that effects of PCBs on brain development may be caused by their ability to affect the thyroid system (Zoeller 2001; Zoeller et al. 2002). Hypothyroidism may also produce menstrual dysfunction, anovulation, and a high incidence of miscarriages (Chiovato et al. 1993).

There are two main types of THs: thyroxine (T₄) and triiodothyronine (T₃), which contain four and three iodine atoms, respectively. T₄ functions as a prohormone for T₃, which is the

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main active hormone. In human blood approximately 0.02% of the T_4 and 0.2% of the T_3 concentrations exist as free molecules (FT_4 and FT_3), while the rest are bound to specific plasma proteins. The most abundant transport proteins are T_4 -binding globulin (TBG), transthyretin (TTR), and albumin (McNabb 1992). Sandau et al. (2000) have documented the presence of the TH-binding proteins TTR and albumin in polar bears.

Vitamin A (retinol) status of marine mammals is also affected by PCBs (Brouwer et al. 1989; Jenssen et al. 2003). Retinol is a fat-soluble and essential vitamin important for the immune system, growth, cell differentiation, vision, and reproduction (Blomhoff 1994; Combs 1992). The toxic effects of PCBs on TH and retinol status appear to be mediated partly by the same molecular mechanism. In plasma, retinol is transported bound to a retinol-binding protein (RBP), whereas T_4 is transported bound to TTR and/or TBG. In the plasma TTR and RBP form a TTR-RBP complex on which both retinol and T_4 are bound and thus transported together. The formation of the TTR-RBP complex is important because it hinders glomerular filtration of RBP (Blomhoff 1994). Because of structural similarities, T_4 and some PCB congeners and/or hydroxylated PCB metabolites (OH-PCBs) compete for binding sites on TTR. When OH-PCBs or PCBs are bound to TTR, the structure of the protein is altered so that the TTR-RBP complex cannot be formed, and plasma levels of both T_4 and retinol decrease (Brouwer et al. 1989). PCBs or their metabolites can also affect THs, TH function, or levels of retinol through other mechanisms (Brouwer et al. 1998; Zoeller 2001): They can directly affect the thyroid gland and can interfere with the binding of THs to the TH receptor. They can also interfere with TH deiodinase activity; with glucuronidation, which causes increased biliary excretion of THs as glucuronic acid conjugates; with sulfotransferases, which are important enzymes in the metabolism of THs; and with thyroid-stimulating hormone.

Skaare et al. (2001) reported that in polar bears from Svalbard, retinol concentrations and the ratio between total T_4 (TT_4) and FT_4 ($TT_4:FT_4$) decreased significantly with increasing plasma concentrations of PCBs. However, this particular study also revealed that several physiologic factors such as sex, age, reproductive status, and nutritional condition also affected retinol and TH status in polar bears. In addition, prevailing seasonal environmental conditions are significant confounding factors with respect to levels of circulating hormones in polar bears (Leatherland and Ronald 1981).

Gestating female polar bears remain in dens throughout the winter, and the cubs are born while the female is in the den. During

the denning period these females with cubs of the year (FWCOY) fast for at least 3 months. Therefore, in the spring they have a nutritional and physiologic status that is different from that of females with yearlings, single females [i.e., females without cubs of the year (FWOCOY)], and males (Derocher et al. 1992; Watts and Hansen 1987). Because sex and reproductive and nutritional status affect TH and retinol status in polar bears (Skaare et al. 2001), it is also possible that the effects of PCBs and other OCs on TH and retinol status may vary as a function of sex and reproductive and nutritional status. In humans, thyroid diseases and hypothyroidism are more common in women than in men, and maternal hypothyroidism can affect the outcome of pregnancy and the neurologic and behavioral abilities of the offspring (Chiovato et al. 1993; Zoeller et al. 2002). Thus, to understand the potential ecologic consequences of PCB-related TH dysfunction in polar bears, it is important to have information on possible sex differences in the effects of PCBs on TH hormone balance and function.

The goal of the present study was to study relationships between PCBs and THs [TT_4 , FT_4 , total T_3 (TT_3), FT_3] and retinol in two groups of female polar bears in different physiologic status (FWCOY and FWOCOY) and in males. All bears were from the Svalbard population of polar bears, in which the concentrations of PCBs in plasma vary between 1.400 and 16.300 ng/g lipid weight (Haave et al. 2003; Olsen et al. 2003). This relatively large variation in PCB exposure among individuals from the same population makes it possible to conduct a correlative study on relationships between PCBs and possibly affected biologic variables such as THs and retinol. To exclude the possible confounding effects due to seasonal variations in physiologic status, we conducted the study during the spring.

Material and Methods

Field sampling. All animals (> 2 years of age) were sampled during the spring (March and April) of 1997 and 1998. The limited period was chosen to avoid the confounding effects of possible temporal changes in the composition of the PCBs in polar bears, as documented in the study population (Henriksen et al. 2001). We obtained samples from 17 FWCOY, 35 FWOCOY, and 29 males. The animals were live-captured at Spitsbergen, Hopen, and Edgeøya, and in the Barents Sea region, east of the border of the Russian economic zone (74–79°N, 16–44°E). The bears were tranquilized by remote injection of a drug-filled dart (Palmer Cap-Chur Equipment, Douglasville, GA, USA) fired from a helicopter (Stirling et al. 1989). Zoletil (Virbac Laboratories, Carros, France) was administered in a solution of 200 mg/mL, at a dose of 5–10 mg/kg body

mass (BM). The condition of each bear was determined by a subjective assessment of subcutaneous fat stores based on palpation of the dorsal and rump fat depots. Bears were assigned a condition index from one to five, with five being the fattest. A vestigial premolar tooth was extracted from all bears for age determination (Calvert and Ramsay 1998). Blood samples were collected from the femoral vein into evacuated containers. The samples were centrifuged and the plasma was pipetted off before they were frozen at -20°C . We estimated the BM of the bears using a morphometric equation (Derocher and Wiig 2002). The Norwegian Experimental Animal Committee approved all the handling methods used during the captures.

PCB analysis. The PCBs were analyzed at the Environmental Toxicology Laboratory at the Norwegian School of Veterinary Science, Oslo, Norway. Because of economic constraints, only six PCB congeners (PCB-99, PCB-118, PCB-153, PCB-156, PCB-180, and PCB-194) were analyzed in plasma samples. These six congeners were chosen because they are representative of several other congeners that accumulate in polar bears (Bernhoft et al. 1997): PCB-99 was closely correlated with PCB-138, PCB-118 with PCB-105, PCB-156 with PCB-157, PCB-180 with PCB-170, and PCB-194 with PCB-206 and PCB-209. Analysis of PCB-153 was included because it is the most abundant PCB congener in polar bears. In an analysis of 28 PCB congeners in 10 polar bear plasma samples, the concentrations of the six congeners analyzed constituted 78% of the total concentration of all 28 congeners (Braathen et al., unpublished data). Because five of the PCB congeners (PCB-99, PCB-153, PCB-156, PCB-180, and PCB-194) were significantly correlated (Pearson correlation: $p < 0.04$, $r \geq 0.39$), these five congeners represent the PCB variable ΣPCB_5 . Because PCB-118 did not correlate with any of the other analyzed congeners (Pearson correlation: $p \geq 0.07$, $r \leq -0.2$), it was thus used as a separate PCB variable.

Plasma samples were weighed and internal standards (PCB-29, PCB-112, and PCB-207) were added before the samples were extracted twice with cyclohexane and acetone and cleaned up with ultra-pure sulfuric acid (Brevik 1978). The extracts were analyzed using a gas chromatograph (Carlo Erba, Milan, Italy) and a software program (Maestro Chromatography Data System, Version 2.4; Chrompack B.V., Middelburg, the Netherlands). This method was described in detail by Bernhoft et al. (1997), with modifications as described by Andersen et al. (2001).

The analytical quality of the laboratory has been approved in several intercalibration tests containing components and matrices relevant to the work, and the laboratory is

accredited by Norwegian Accreditation as a testing laboratory for OCs as described by Sørmo et al. (2003).

Detection limits for individual compounds were determined as three times the noise level. Quantification was carried out with response factors from PCB standards made from individual congeners obtained from Promochem GmbH (Wesel, Germany). We performed the quantification using PCB-29, PCB-112, and PCB-207 as internal standards in each sample. Detection limits for individual PCB congeners were between 0.005 and 0.009 ng/g wet weight (ww). Recoveries and coefficient of variation (CV) of individual PCB congeners in spiked sheep blood varied from 87 to 107% (percent CV = 5–11, $n = 58$) which were in the acceptable range set by the laboratory quality control system. Reproducibility was tested continuously by analyzing the PCB levels in the laboratory's own reference sample material (seal blubber), and the results were within $102.6 \pm 11.5\%$ (mean \pm SD; $n = 22$).

Retinol analysis. Retinol concentrations were determined in blood plasma using HPLC with fluorescence detection (LKB-Bromma 2155 HPLC-column oven, Pharmacia LKB Autosampler 2157, and Fluorescence Detector 2144; Pharmacia LKB, Uppsala, Sweden) (Jenssen et al. 2003; Shearer 1986). Retinol was extracted using ethanol (96%), and *n*-hexane was added to the mixture. The extracts were then evaporated to dryness and the residues were dissolved once more in the mobile phase (99.9% HPLC-methanol). Retinol (vitamin A1) was quantified by peak integration in relation to standards of retinol (Sigma R-7632; Sigma-Aldrich Norway AS, Oslo, Norway) in ethanol. All plasma levels of retinol are presented on a wet weight basis.

Because of photoreactivity of retinol, all sample handling and experimental steps were carried out in subdued light. All determinations were made in duplicate, and the sensitivity of the assay was improved so the plasma samples could be analyzed in duplicate with a CV < 15%. In the few cases where the CV exceeded 15%, new duplicates were analyzed. We tested reproducibility by adding a duplicate control sample to each series of five samples. The control samples consisted of plasma from one polar bear from which extra blood was collected. The reproducibility was considered acceptable (mean = 196 μ g/L, SE = 6.46, $n = 81$). The calibration graph of peak ratios of all-trans retinol was linear over the range of 0–800 μ g/L ($r^2 = 0.998$, $p < 0.0001$; simple regression analysis).

Hormone analysis. We used radioimmunoassay (RIA) to determine TH concentrations. We used commercially available RIA kits with coated tubes for the analyses (Coat-A-Count Total T_4 , Coat-A-Count Free T_4 , Coat-A-Count Total T_3 , and Coat-A-Count

Free T_3 ; Diagnostic Product Corporation, Los Angeles, CA, USA). The amount of the bound radioactive antigen was quantified using a gamma counter (Cobra Auto-Gamma; Packard Instrument Company, Dowers Grove, IL, USA).

Plasma samples were analyzed in duplicate and the mean values were used in the statistical treatment of the results. The TH concentrations with CVs > 20% between the duplicate analyses were omitted; therefore, the sample size with respect to the different TH variables varies. Control samples composed of plasma from several individual polar bears were also run for each 10 samples to test the variation of the analyses. The interassay percent CV for the different hormones were as follows: for TT_4 : high concentration, 9.7% ($n = 12$); medium concentration, 8.6% ($n = 8$); low concentration, 3.6% ($n = 3$); for FT_4 : high concentration, 20% ($n = 10$); medium concentration, 28% ($n = 8$); low concentration, 11.7% ($n = 8$); and for TT_3 : high concentration, 6.0% ($n = 11$); medium concentration, 7.8% ($n = 12$); low concentration, 4.0% ($n = 5$). Interassay tests of FT_3 could not be conducted, but the instructions in the Coat-A-Count Free T_3 kit stated the following values: high concentration, 5.0%; medium concentration, 6.8%; and low concentration, 8.8%. Also, according to the kit instructions, the cross-reactivity of FT_3 with T_4 was 0.0008%, and cross-reactivity of FT_4 with T_3 was 0.001%. For TT_3 and TT_4 the values were given only as low and very low, respectively.

Statistics. All statistical analyses and calculations were conducted using the SPSS statistical software, Version 10.0, standard version (SPSS Inc., Chicago, IL, USA). Data were analyzed for normality using the Shapiro-Wilcoxon test when n was < 50 and the Kolmogorov-Smirnov test when n was > 50.

Variables not normally distributed were \log_{10} transformed. This applied to age, BM, PCB concentrations, TT_4 , TT_3 , $TT_4:TT_3$, $FT_4:FT_3$, and $TT_3:FT_3$. The statistical significance level was set at $p \leq 0.05$. In statistical analyses, we used PCB-118, Σ PCB₅, plasma fat, and age as predictor variables. There was a high degree of colinearity between BM and age (Pearson correlation: $r > 0.61$, $p < 0.001$). Based on previous reports that age affects TH and retinol status in polar bears (Skaare et al. 2001), age and not BM was chosen as the predictor variable of these two. The effects of the predictor variables were studied on the variables retinol, TT_4 , FT_4 , TT_3 , FT_3 , and the hormone ratios $TT_4:FT_4$, $TT_3:FT_3$, $TT_4:TT_3$, and $FT_4:FT_3$ using analysis of variance (ANOVA) and Tukey's post hoc test. Backward multiple linear regression was used to determine which of the predictor variables could best describe the variation in the retinol and THs.

When studying relationships between PCBs and the dependent plasma variables in blood, we used wet weight concentration of PCBs because this probably represents the most relevant exposure concentration.

Results

Data on relevant biologic variables in polar bears from the three groups are summarized in Table 1. There were no condition data for eight bears captured in 1997. BM differed significantly between the three groups (ANOVA: $F = 19.22$, $p < 0.0001$). Males had significantly higher BM than both FWCOY and FWOCOY (Tukey's post hoc test: $p < 0.0001$ for both groups), whereas there were no differences between FWCOY and FWOCOY. Plasma fat also differed between the groups (ANOVA: $F = 7.36$, $p = 0.001$); FWOCOY had significantly higher levels of plasma fat

Table 1. Age, BM, and plasma fat from polar bears, Svalbard, Norway.

	FWCOY	FWOCOY	Males
Age (years)			
No.	17	35	29
Mean \pm SD	8.8 \pm 4.2	11.5 \pm 6.5	11 \pm 6
Range	4–21	2–26	2–25
Median	7	11	10
95% CI	6.7–11.0	9.3–13.7	8.8–13.3
BM (kg)			
No.	16	35	29
Mean \pm SD	176 \pm 20	179 \pm 31	303 \pm 106 ^a
Range	147–214	94–245	67–459
Median	173	178	316
95% CI	165–187	168–190	263–343
Plasma fat (%)			
No.	17	35	29
Mean \pm SD	1.14 \pm 0.21	1.19 \pm 0.28 ^b	0.97 \pm 0.18
Range	0.76–1.50	0.49–1.66	0.69–1.34
Median	1.10	1.16	0.96
95% CI	1.03–1.24	1.09–1.29	0.90–1.04

CI, confidence interval.

^a $p \leq 0.0001$ by Tukey's post hoc test compared with FWCOY and FWOCOY; FWCOY and FWOCOY were not significantly different. ^b $p \leq 0.001$ by Tukey's post hoc test compared with males; FWCOY and FWOCOY were not significantly different.

than males (Tukey's post hoc test: $p = 0.001$), but there were no differences in plasma fat between FWCOY and FWOCOY. Age was not significantly different between the three different groups (ANOVA: $F = 0.42$, $p = 0.660$).

ΣPCB_5 in plasma of the polar bears ranged from 16.7 to 203.2 ng/g ww (Table 2). We observed no effect of age on ΣPCB_5 or PCB-118 in any of the groups (Pearson correlation: $p > 0.05$). Because of a high degree of collinearity between BM and age (Pearson correlation: $r > 0.61$, $p < 0.001$), we excluded BM from further statistical analyses. We found no differences in concentrations of ΣPCB_5 or PCB-118 between the three groups (ANOVA: $p > 0.05$) (Table 2).

We found significant differences in plasma concentrations of TT_4 , FT_4 , and TT_3 between the groups (ANOVA: $F = 8.13$, $p = 0.001$; $F = 5.82$, $p = 0.005$; and $F = 6.73$, $p = 0.002$, respectively; Table 3). TT_4 , FT_4 , TT_3 were higher in FWOCOY than in males (Tukey's post hoc: $p = 0.001$; $p = 0.003$; and $p = 0.003$, respectively). Also, TT_3 was higher in FWOCOY than in FWCOY (Tukey's post hoc: $p = 0.034$). There were no differences in FT_3 between the groups (ANOVA: $F = 2.37$, $p = 0.11$) or in plasma retinol concentrations between the three groups (ANOVA: $F = 0.60$, $p = 0.550$; Table 3).

Relationships between PCBs and THs and retinol. In FWCOY the variation in FT_4 was best explained by ΣPCB_5 , giving a negative correlation ($F = 6.33$, $r^2_{\text{adj}} = 0.35$, $p = 0.033$; Figure 1A). The variation in FT_3 was explained by both ΣPCB_5 and age ($F = 10.90$, $r^2_{\text{adj}} = 0.80$, $p = 0.042$). After partial correlation correcting for age, ΣPCB_5 accounted for 80% of the variation in FT_3 , giving a negative correlation ($r^2 = 0.80$, $p = 0.042$; Figure 1B). None of the predictors could explain the variation in retinol, TT_4 , or TT_3 . The variation in TT_4 : TT_3 was explained by ΣPCB_5 and plasma fat ($F = 22.08$, $r^2_{\text{adj}} = 0.88$, $p = 0.007$). Partial correlation correcting for plasma fat showed

that ΣPCB_5 accounted for 92% of the variation in TT_4 : TT_3 , giving a negative correlation ($r^2 = 0.92$, $p = 0.003$; Figure 1C). The variation in TT_4 : FT_4 , FT_4 : FT_3 , and TT_3 : FT_3 could not be explained by any of the independent variables.

In FWOCOY the variation in TT_4 was best explained by age and ΣPCB_5 ($F = 4.82$, $r^2_{\text{adj}} = 0.21$, $p = 0.016$). Partial correlation correcting for age gave a negative correlation between ΣPCB_5 and TT_4 ($r^2 = 0.14$, $p = 0.044$; Figure 2A). The variation in FT_3 was best explained by PCB-118, giving a positive correlation ($F = 5.02$, $r^2_{\text{adj}} = 0.21$, $p = 0.042$; Figure 2B). None of the dependent variables could explain the variation in retinol, FT_4 , or TT_3 . In FWOCOY the variation in TT_3 : FT_3 was best explained by both PCB-118 and ΣPCB_5 ($F = 7.42$, $p = 0.007$, $r^2_{\text{adj}} = 0.46$). Partial correlation correcting for PCB-118 gave a positive correlation between ΣPCB_5 and

TT_3 : FT_3 ($r^2 = 0.31$, $p = 0.031$; Figure 2C), whereas correcting for ΣPCB_5 gave a negative correlation between PCB-118 and TT_3 : FT_3 ($r^2 = 0.26$, $p = 0.050$; Figure 2D). The variation in TT_4 : TT_3 was best explained by ΣPCB_5 and age ($F = 3.87$, $r^2_{\text{adj}} = 0.20$, $p = 0.037$), but partial correlation correcting for age gave no significant relationship. The variation in TT_4 : FT_4 and FT_4 : FT_3 could not be explained by any of the independent variables.

In males the variation in TT_4 was best explained by age, giving a negative correlation ($F = 5.17$, $r^2_{\text{adj}} = 0.17$, $p = 0.034$). The variation in TT_3 was best explained by the two predictors plasma fat and age ($F = 6.14$, $r^2_{\text{adj}} = 0.34$, $p = 0.009$). Partial correlation correcting for age showed no correlation between plasma fat and TT_3 , whereas correcting for plasma fat resulted in a negative relationship between age and TT_3 ($r^2 = 0.24$, $p = 0.028$). The variation in FT_3 was best

Table 3. Concentrations of THs and retinol in plasma from polar bears, Svalbard, Norway.

	TT_4 (nmol/L)	FT_4 (nmol/L)	TT_3 (nmol/L)	FT_3 (nmol/L)	Retinol ($\mu\text{g/L}$)
FWCOY					
No.	9	11	14	6	17
Mean \pm SD	15.3 \pm 5.18	4.38 \pm 1.77	1.26 \pm 0.26	1.26 \pm 0.75	181 \pm 51.5
Range	9.74–27.1	2.22–7.23	0.65–1.61	0.41–2.33	105–288
Median	14.2	3.96	1.30	1.02	177
95% CI	11.3–19.3	3.19–5.57	1.11–1.40	0.48–2.05	154–207
FWOCOY					
No.	30	28	28	16	22
Mean \pm SD	18.5 \pm 5.44 ^a	5.20 \pm 1.80 ^b	1.58 \pm 0.43 ^{c,d}	1.42 \pm 0.56	201 \pm 62.4
Range	10.7–31.5	2.25–9.85	0.89–2.56	0.14–2.41	88.0–332
Median	17.0	5.12	1.46	1.53	202
95% CI	16.4–20.5	4.50–5.90	1.42–1.75	1.12–1.72	173–228
Males					
No.	22	23	21	11	15
Mean \pm SD	13.5 \pm 3.25	3.46 \pm 1.84	1.23 \pm 0.36	0.95 \pm 0.38	183 \pm 73.3
Range	8.87–22.7	0.73–9.74	0.68–2.31	0.29–1.41	56.0–301
Median	13.1	3.19	1.24	0.92	171
95% CI	12.1–14.9	2.67–4.26	1.07–1.39	0.70–1.20	142–224

CI, confidence interval.

^a $p \leq 0.001$ by Tukey's post hoc test compared with males; FWCOY and FWOCOY were not significantly different. ^b $p \leq 0.003$ by Tukey's post hoc test compared with males; FWCOY and FWOCOY were not significantly different. ^c $p \leq 0.003$ by Tukey's post hoc test compared with males; FWCOY and FWOCOY were not significantly different. ^d $p \leq 0.034$ by Tukey's post hoc test compared with FWCOY.

Table 2. Concentrations of PCB congeners in plasma from FWCOY, FWOCOY, and male polar bears, Svalbard, Norway.

	PCB-99 (ng/g ww)	PCB-118 (ng/g ww)	PCB-153 (ng/g ww)	PCB-156 (ng/g ww)	PCB-180 (ng/g ww)	PCB-194 (ng/g ww)	ΣPCB_5 (ng/g ww)
FWCOY (n = 17)							
Mean \pm SD	9.01 \pm 4.68	0.23 \pm 0.10	41.1 \pm 23.1	1.22 \pm 0.68	27.6 \pm 15.3	4.91 \pm 3.80	83.9 \pm 46.6
Range	2.00–19.97	0.10–0.45	7.69–102.4	0.40–2.78	5.6–60.0	1.45–18.10	17.1–203.2
Median	8.02	0.21	39.1	0.97	25.8	3.94	80.45
95% CI	6.60–11.42	0.18–0.28	29.3–53.0	0.87–1.57	19.7–35.5	2.95–6.86	59.93–107.8
FWOCOY (n = 35)							
Mean \pm SD	8.50 \pm 4.60	0.31 \pm 0.14	37.8 \pm 22.7	1.05 \pm 0.51	24.8 \pm 13.1	5.42 \pm 2.76	77.6 \pm 41.9
Range	2.16–18.70	0.09–0.69	8.4–95.3	0.34–2.87	4.0–65.2	1.34–11.91	16.7–190.2
Median	7.84	0.29	36.7	1.05	23.8	4.82	74.8
95% CI	6.92–10.08	0.26–0.36	30.0–45.6	0.87–1.22	20.3–29.3	4.47–6.37	63.2–92.0
Males (n = 29)							
Mean \pm SD	7.41 \pm 5.54	0.33 \pm 0.19	34.6 \pm 22.0	1.10 \pm 0.60	23.0 \pm 13.6	6.19 \pm 6.52	72.3 \pm 43.6
Range	1.71–27.44	0.11–0.93	9.5–102.6	0.31–2.71	7.8–56.8	1.51–35.69	25.2–186.0
Median	6.04	0.27	29.7	1.01	17.3	3.69	63.6
95% CI	5.30–9.52	0.26–0.40	26.2–43.0	0.87–1.32	17.8–28.2	3.70–8.67	55.7–88.9

CI, confidence interval.

explained by ΣPCB_5 , giving a negative correlation ($F = 13.90$, $r^2_{adj} = 0.56$, $p = 0.005$; Figure 3A). None of the independent variables could explain the variation in FT_4 . In males the variation in $\text{TT}_4:\text{FT}_4$ was best explained by PCB-118 , plasma fat, and age ($F = 3.97$, $r^2_{adj} = 0.34$, $p = 0.031$), but partial correlation correcting for age and plasma fat did not yield any significant relationships. The variation in $\text{FT}_4:\text{FT}_3$ was best explained by ΣPCB_5 and PCB-118 ($F = 14.94$, $r^2_{adj} = 0.76$, $p = 0.003$). Partial correlation correcting for PCB-118 gave a positive correlation between ΣPCB_5 and $\text{FT}_4:\text{FT}_3$ ($r^2 = 0.78$, $p = 0.002$; Figure 3B), whereas correcting for ΣPCB_5 gave a negative correlation between PCB-118 and $\text{FT}_4:\text{FT}_3$ ($r^2 = 0.53$, $p = 0.026$; Figure 3C). None of the dependent variables could explain the variation in $\text{TT}_4:\text{TT}_3$ and $\text{TT}_3:\text{FT}_3$. Plasma fat was the best predictor for retinol in males, with a positive correlation ($F = 19.15$, $r^2_{adj} = 0.57$, $p = 0.001$).

Discussion

PCBs affected five TH variables in female polar bears (TT_4 , FT_4 , FT_3 , $\text{TT}_3:\text{FT}_3$, $\text{TT}_4:\text{TT}_3$) but only two TH variables in males (FT_3 , $\text{FT}_4:\text{FT}_3$). In humans, thyroid diseases are more common in women than in men, and women are more likely to develop hypothyroidism, both overt and subclinically (Chiovato et al. 1993; Krueger et al. 2001; Larsen 1992). Thus, it is possible that the higher incidence of TH imbalance found in female polar bears compared with males could reflect the greater susceptibility of female polar bears to TH-related effects of PCBs. To our knowledge, this is the first report of a possible sex difference in PCB-related effects on TH status in any mammals.

Exposure to PCBs has been associated with cognitive and behavioral changes (Jacobson et al. 1990; Schantz 1996; Sher et al. 1998), and it has been suggested that the effects of

PCBs on brain development may be attributable, at least in part, to their ability to affect the thyroid system (Zoeller 2001; Zoeller et al. 2002). We have little information on normal plasma levels of THs in polar bears. The levels

of TT_4 and TT_3 we found are similar to those previously reported in polar bears from Svalbard (Skaare et al. 2001). In polar bears from Canada, which generally have lower PCB levels, both higher and lower levels than those

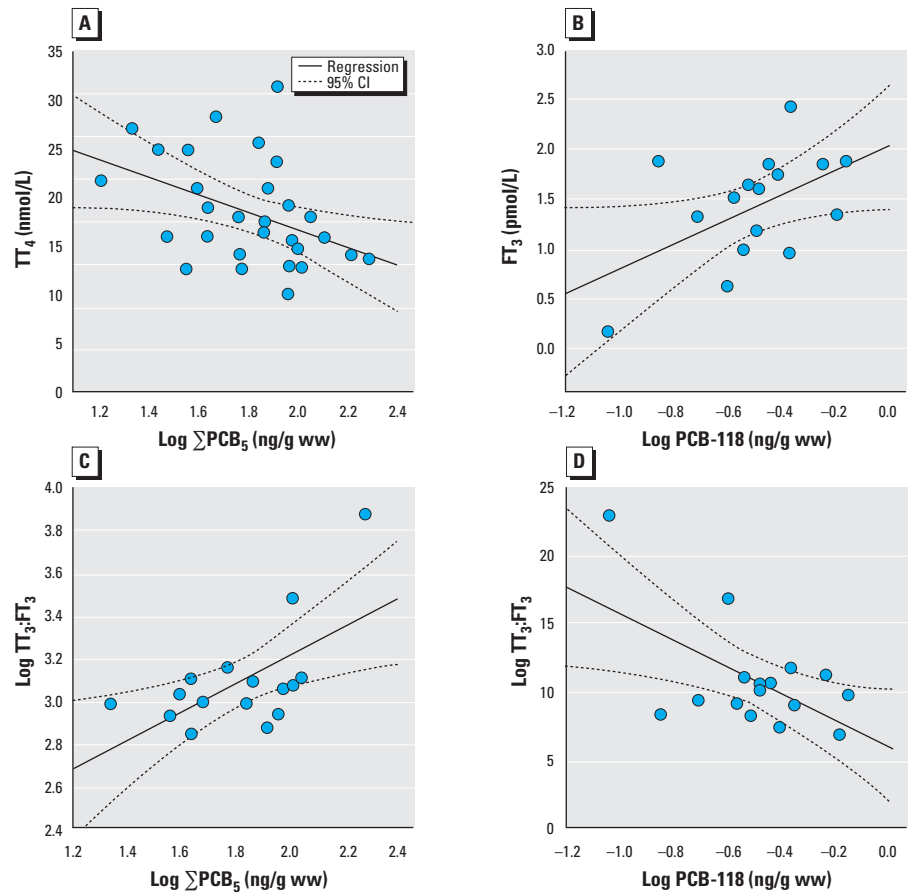


Figure 2. Thyroid hormone concentrations in relation to ΣPCB_5 and PCB-118 in FWOCOY, Svalbard, Norway. CI, confidence interval. (A) Correlation of TT_4 and $\text{log } \Sigma\text{PCB}_5$ ($y = -9.1x + 35$; $r^2 = 0.14$; $p = 0.044$; $n = 30$); r^2 and p -values were corrected for influence by age. (B) Correlation of FT_3 and PCB-118 ($y = 1.3x + 2.0$; $r^2 = 0.21$; $p = 0.042$; $n = 16$). (C) Correlation of $\text{log } \text{TT}_3:\text{FT}_3$ and $\text{log } \Sigma\text{PCB}_5$ ($y = 0.66x + 1.9$; $r^2 = 0.31$; $p = 0.031$; $n = 16$); r^2 and p -values were corrected for influence from PCB-118 . (D) Correlation of $\text{log } \text{TT}_3:\text{FT}_3$ and $\text{log } \text{PCB-118}$ ($y = -0.63x + 2.8$; $r^2 = 0.26$; $p = 0.050$; $n = 16$); r^2 and p -values are corrected for influence from ΣPCB_5 .

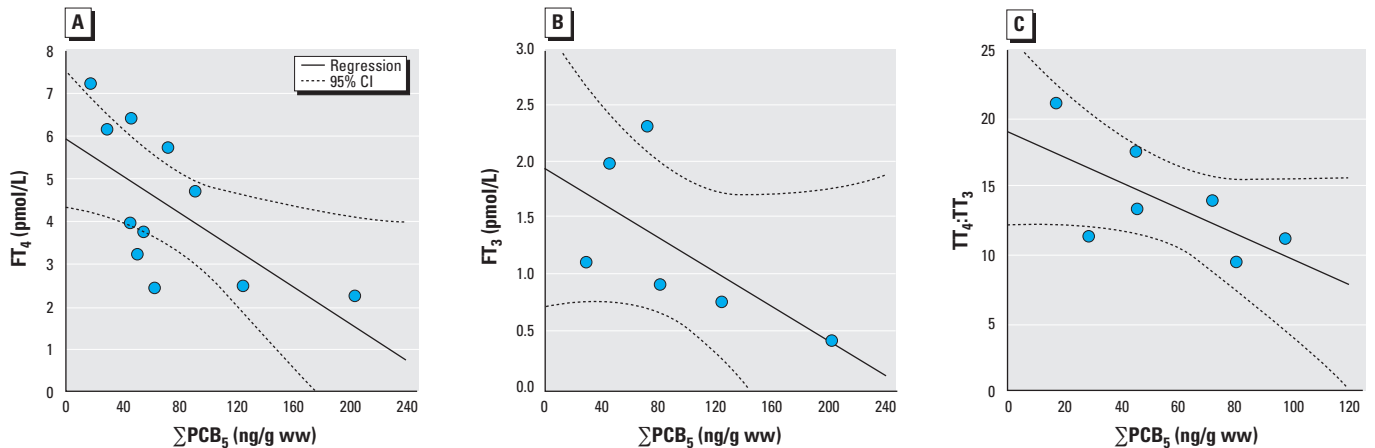


Figure 1. Thyroid hormone concentrations in relation to ΣPCB_5 in FWOCOY, Svalbard, Norway. CI, confidence interval. (A) FT_4 ($y = -0.4x + 5.9$; $r^2_{adj} = 0.35$; $p = 0.033$; $n = 11$). (B) FT_3 ($y = 7.6 \times 10^{-3}x + 2.0$; $r^2 = 0.80$; $p = 0.042$; $n = 6$); r^2 and p -values were corrected for influence by age. (C) $\text{TT}_4:\text{TT}_3$ ($y = -0.09x + 18.9$; $r^2 = 0.92$; $p = 0.003$; $n = 7$); r^2 and p -values were corrected for influence from plasma fat.

found in the present study have been reported (Leatherland and Ronald 1981; Sandau 2000). Because knowledge of normal baseline TH plasma levels in polar bears is very sparse, it is not possible to judge whether any of the polar bears in the present study suffered from hypothyroidism. However, the negative relationship we found between PCBs and THs should raise concern about the possible effects of PCB exposure on the learning ability and behavior of polar bears at Svalbard.

The actions of THs are mediated by nuclear TH receptors that have their highest affinity for FT₃ (McNabb 1995). However, most studies on PCB-related effects on TH function and status have focused on TT₄, TT₃, and to some extent on FT₄ (Brouwer et al. 1998; Rolland 2000). Very few studies have examined FT₃. In the present study, FT₃ was affected in all three groups studied. In a previous study on polar bears from Svalbard, no relationships between PCBs and FT₃ or TT₃ were found (Skaare et al. 2001). The difference in results between the study of Skaare et al. (2001) and the present study could be because we studied the effects within physiologically comparable groups of animals in this study.

The negative relationships between Σ PCB₅ and FT₃ we found are in accordance with results reported in Larga seals (*Phoca larga*) (Chiba et al. 2001). Significantly lower plasma concentrations of FT₃ have also been found in gray seal pups from the highly polluted Baltic Sea compared with a much cleaner reference site at the Norwegian coast (Sørmo et al., unpublished data). In an experimental study in mink (*Mustela vison*) exposed to PCB-contaminated carp (*Cyprinus carpio*), FT₃ and TT₃ decreased with increasing 2,3,7,8-tetrachlorodibenzodioxin equivalent (TEQ) values in their food (Heaton et al. 1995). Furthermore, in second-grade schoolchildren from Germany, there was a negative

correlation between Σ PCB (PCB-138, PCB-153, PCB-180, PCB-183, PCB-187) and FT₃ (Osius et al. 1999). However, it must be noted that there are other studies that report no relationships between PCBs and FT₃. In gray seal pups from the United Kingdom, Hall et al. (1998) found no correlation between Σ PCB (sum of 26 congeners) and FT₃. However, in that particular study, the range in the Σ PCB levels was quite low (1,432–2,398 ng/g lipid), and the levels may have been below the threshold level for effect on FT₃. In fishermen's wives from the Swedish east coast (23–62 years of age) who consumed a high level of OC-contaminated fatty fish from the Baltic Sea, Hagmar et al. (2001) observed no effects on FT₃, even though a negative relationship was reported between PCB-153 and TT₃.

In the present study, we found a positive relationship between PCB-118 and FT₃ in FWOCOY. PCB-118 binds to some extent to the aryl hydrocarbon (Ah) receptor and mediates so-called dioxin-like toxicity; therefore, the negative effects of PCBs on TH status in polar bears are not mediated via the Ah receptor. Polar bears metabolize PCB-118 to a greater extent than the other congeners examined in this study (Letcher et al. 1996; Norstrom and Muir 1994). Induction of cytochrome P450 enzymes caused by high levels of PCBs will therefore decrease PCB-118 levels more than the other, more-persistent congeners (Brown et al. 1989). This may explain why the relationships between PCB-118 and THs were positive, whereas those between Σ PCB₅ and THs were negative (Figures 2 and 3).

In the present study, T₄ seems to be affected to a lesser degree than T₃. Negative relationships between PCBs and T₄ have been reported in numerous studies on experimental rodent models (Brouwer et al. 1998). In wildlife studies there are indications that PCBs

depress plasma levels of T₄ in seals (Beckmen et al. 1997; Brouwer et al. 1989; Reijnders 1986). However, in mink exposed to PCB-contaminated carp, TT₄ and FT₄ increased with increasing TEQ levels in their food (Heaton et al. 1995).

The levels of THs differed between the sexes; TH levels were generally higher in females than in males (Table 3). This result agrees with previous studies of THs in polar bears (Leatherland and Ronald 1981; Skaare et al. 2001). However, in polar bears captured in Resolute Bay in the Canadian Arctic, only T₃ levels were significantly higher in females than in males (Sandau 2000). In the present study, we also found significantly higher levels of THs in FWOCOY compared with FWCOY. Because fasting is associated with a decrease in TH levels (Azizi et al. 1979; Tomasi 1991; Tomasi et al. 1998), it is possible that FWCOY had decreased levels of THs because of their recent fast. It should be noted, however, that discrepancy in TH concentrations between studies could be due to the use of different analytical procedures.

There are several mechanistic explanations for the toxic effects of PCBs on TH status (Brouwer et al. 1998; Cheek et al. 1999; DeVito et al. 1999; Sher et al. 1998; Zoeller et al. 2002). PCBs or their metabolites can directly affect the thyroid gland: they can interfere with the binding of THs to plasma transport proteins or the TH receptor; with deiodination, glucuronidation, iodothyronine sulfation; and with thyroid-stimulating hormone. It is therefore important to note that TTR, albumin, and OH-PCBs have been detected in polar bear plasma (Sandau et al. 2000). This particular study also documented high concentrations of other OC metabolites that have OH groups with adjacent halogenated substituents that can bind to TTR. For example, the binding capacity

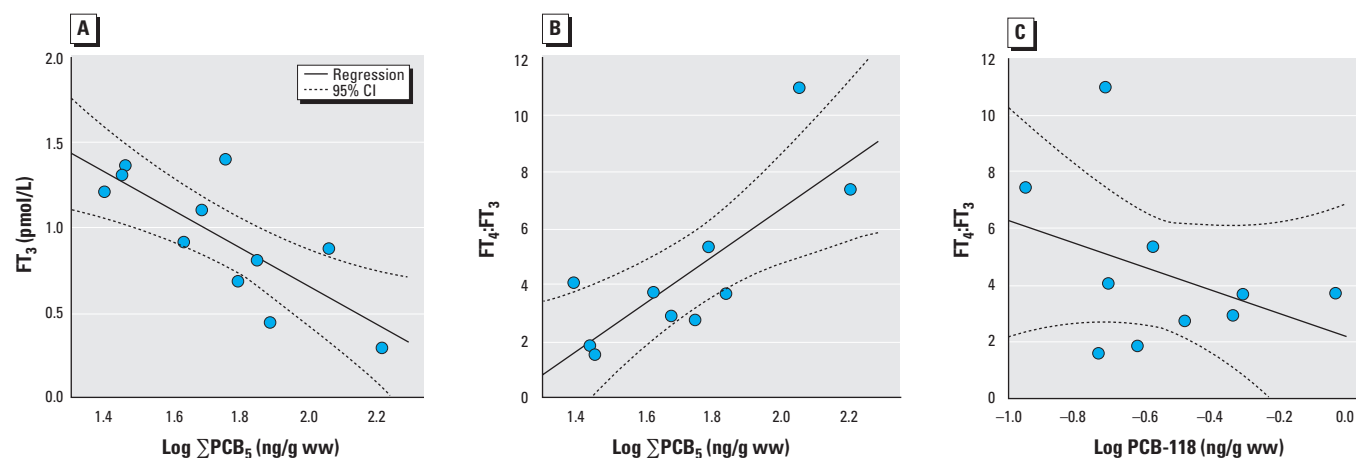


Figure 3. Thyroid hormone concentrations in relation to Σ PCB₅ and PCB-118 in male polar bears, Svalbard, Norway. CI, confidence interval. (A) Correlation of FT₃ and log Σ PCB₅ ($y = -1.1x + 2.9$; $r^2_{\text{adj}} = 0.56$; $p = 0.005$; $n = 11$). (B) Correlation of FT₄:FT₃ and log Σ PCB₅ ($y = 8.3x - 10.0$; $r^2 = 0.78$; $p = 0.002$; $n = 10$); r^2 and p -values are corrected for influence by PCB-118. (C) Correlation of FT₄:FT₃ and log PCB-118 ($y = -4.1x + 2.2$; $r^2 = 0.53$; $p = 0.026$; $n = 10$); r^2 and p -values are corrected for influence from Σ PCB₅.

of 4'-OH-3,3',4,5'-PCB (OH-PCB-79) toward TTR was four times greater than that of T₄ (Lans et al. 1994). Thus, it is possible that the apparent higher susceptibility of female polar bears to PCB-related TH imbalance compared with males may be due to higher concentrations of OH-PCBs in females, as reported in polar bears from North America (Sandau et al. 2000).

We found no correlations between PCBs and plasma retinol concentrations in the present study. These results are in contrast to the other study on Svalbard polar bears (Skaare et al. 2001) in which such a relationship was documented. We examined effects within standardized and comparable groups with respect to sex, reproductive status, and season of sampling. Several possible intrinsic and extrinsic confounding effects were thus eliminated. Retinol is a fat-soluble vitamin; therefore free retinol is likely to be associated with plasma fat. However, although males had lower levels of plasma fat than females, the retinol levels did not differ between the three groups (males, FWCOY, FWOCOY). Even so, retinol was positively correlated to plasma fat in males, but not in the two female groups. It is possible that differences in plasma lipid composition between the sexes may contribute to sex differences in retinol homeostasis relative to plasma lipids. The plasma retinol concentrations we found are within the range previously reported for polar bears at Svalbard (Skaare et al. 2001).

In the Svalbard population of polar bears, a relative scarcity of FWCOY \geq 16 years of age has been reported (Derocher et al. 2003). This could mean that reproduction in Svalbard polar bears may be impaired (Derocher et al. 2003). Although there is no evidence that the female polar bears in the present study were suffering from clinical hypothyroidism, it should be noted that maternal hypothyroidism can produce menstrual dysfunction, anovulation, high incidence of miscarriage, maternal complications, and congenital malformations (Chiovato et al. 1993). Haave et al. (2003) have documented that in female polar bears from Svalbard, progesterone levels were positively correlated with PCBs. Increased levels of progesterone may disturb the normal reproductive cycle of females and thus hinder successful mating. Thus, it is functionally possible that PCB exposure may affect reproduction of Svalbard polar bears via disruption of both progesterone levels and TH levels. However, further studies are needed to clarify the hypothesized link between hormone disruption caused by PCBs or other OCs and reproductive dysfunction in polar bears.

Conclusions

Although the relationships found between PCBs and THs found in polar bears is not

evidence per se of a direct cause-effect association, we conclude that the present study indicates that PCBs interfere with TH balance in polar bears and that female polar bears seem to be more susceptible than males. Furthermore, T₃ seems to be affected by PCBs to a larger degree than T₄. There is a clear need to focus on possible functional effects of the apparent PCB-induced TH imbalance in polar bears.

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