Polychlorinated Biphenyls and Reproductive Hormones in Female Polar Bears at Svalbard

Marte Haave,[1] Erik Ropstad,[2] Andrew E. Derocher,[3] Elisabeth Lie,[4] Ellen Dahl,[3] Øystein Wiig,[5] Janneche U. Skaare,[4,6] and Bjørn Munro Jenssen[1]

[1]Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway; [2]Department of Reproduction and Forensic Medicine, Norwegian School of Veterinary Science, Oslo, Norway; [3]Norwegian Polar Institute, Tromsø, Norway; [4]National Veterinary Institute, Oslo, Norway; [5]Zoological Museum, University of Oslo, Oslo, Norway; [6]The Norwegian School of Veterinary Science, Oslo, Norway

High concentrations of polychlorinated biphenyls (PCBs) in polar bears from Svalbard have increased concern for that population's reproductive health. We examined whether there were associations between the plasma concentrations of PCBs and reproductive hormones [progesterone (P_4) and 17β-estradiol (E_2)] in free-living female polar bears from Svalbard. Concentrations of P_4 depended on reproductive status, and concentrations were lowest in females with offspring—females with cubs and females with yearlings. In these females, the P_4 concentrations were positively correlated with plasma ΣPCBs (sum of all analyzed polychlorinated biphenyl congeners) concentrations. The ΣPCBs concentrations explained 27% of the variation in the P_4 concentrations. There were no correlations between ΣPCBs and E_2 and cortisol in any of the groups of polar bears, or between ΣPCBs and P_4 in single polar bears. Although the ΣPCBs–P_4 relationship in female polar bears with offspring is not evidence per se of a direct cause–effect association, the results indicate that PCBs may affect levels of P_4 in polar bear females. There is a clear need to further assess the hormone balance and population health of polar bears at Svalbard. *Key words:* **Arctic, Barents Sea, endocrine disruption, endocrine disruptors, estradiol, pollution, population, progesterone, Svalbard.** *Environ Health Perspect* **111:431–436 (2003). doi:10.1289/ehp.5553 available via** *http://dx.doi.org/* **[Online 21 November 2002]**

Long-range transport of persistent organic compounds by air and ocean currents from industrialized areas has produced high levels of these pollutants in top predators in the Svalbard area, Barents Sea, and Kara Sea (Andersen et al. 2001; Norstrom et al. 1998; Skaare et al. 2000). The cold Arctic environment acts as a sink where the semivolatile, persistent contaminants are deposited and enter the marine food web (Oehme 1991). Polychlorinated biphenyls (PCBs), chlordanes (*trans*-nonachlor, oxy-, *cis*-, and *trans*-chlordane), 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethylene (DDE), hexachlorobenzene (HCB), hexachlorocyclohexanes (α- and β-HCHs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) are among the organochlorines (OCs) detected (Bernhoft et al. 1997; Norstrom and Muir 1994; Norstrom et al. 1998; Oehme 1991). These OC pollutants are lipophilic and resistant to metabolic degradation and excretion, they accumulate in lipid-rich tissue, and they are magnified with increasing trophic level in the food web (Muir et al. 1992; Norstrom and Muir 1994). The main prey of polar bears (*Ursus maritimus*) are ringed seals (*Phoca hispida*), bearded seals (*Erignathus barbatus*), and harp seals (*Pagophilus groenlandicus*) (Lønø 1970; Smith 1980), and polar bears are thus the ultimate apex predator in the arctic marine ecosystems. Polar bears primarily consume the blubber of their prey (Stirling and McEwan 1975) and are therefore especially at risk of accumulating lipophilic compounds.

PCBs are the predominant OC compounds detected in polar bears, and PCB concentrations in adult female polar bears from Svalbard range from 2,200 to 33,000 ng/g lipid in blood and from 6,200 to 33,000 ng/g lipid in adipose tissue (Bernhoft et al. 1997; Norstrom et al. 1998). PCBs are among the OCs that have been given special attention with respect to reproductive toxicity and endocrine disruptive potential. Reijnders (1986) reported that common seals (*Phoca vitulina*) fed PCB-contaminated fish had lower reproductive rates than the control group fed relatively unpolluted fish. Furthermore, it has been reported that PCB exposure before or during gestation led to fetal growth retardation and fetal mortality in mink (*Mustela vison*) (Backlin et al. 1998; Kihlstrom et al. 1992). PCBs have also been shown to produce trophoblastic lesions in mink (Backlin et al. 1998) and are believed to be the cause of uterine occlusions in seals (Bergman and Olsson 1985; Helle et al. 1976). The biochemical mechanisms by which PCBs exert their reproductive effects are poorly understood, but PCBs may affect several steps in the reproductive cycle from ovulation and fertilization to implantation and fetal development (Backlin et al. 1998; Kihlstrom et al. 1992). It is also known that PCB congeners may act both estrogenic and antiestrogenic (Colborn et al. 1993; Connor et al. 1997; Nesaretnam et al. 1996). These factors suggest that PCBs may have the potential to interfere with reproduction by disturbing the steroid-hormone balance. The high concentrations of PCBs reported in polar bears from the Svalbard area have thus resulted in growing concerns for the population's reproductive health (Skaare et al. 2000; Wiig et al. 1998).

The aim of the present study was to study if PCB exposure in free-living female polar bears from Svalbard may affect their reproductive hormone concentrations. Thus, we tested to what extent PCB plasma levels in different reproductive groups of female polar bears could explain variations in plasma concentrations of the reproductive hormones progesterone (P_4) and 17β-estradiol (E_2), and cortisol.

Materials and Methods

This study is part of a project designed to study levels, tissue distribution, and possible effects of OCs in polar bears in the Norwegian Arctic. Blood samples for OC analyses were collected from 360 male and female polar bears of different ages. We used a subsample consisting of 86 adult female (≥ 4 years of age) polar bears to examine the possible effects of PCBs on reproductive hormones. These females were live-captured in the spring (27 March–8 May) from 1995 to 1998. The samples were collected at Svalbard, Hopen, Edgeøya, and in the Barents Sea region, east to the border of the Russian economic zone (74–79°N and 16–44°E).

The animals were captured by remote injection of a drug-filled dart (Palmer Cap-Chur Equipment, Douglasville, Georgia, USA) fired from a helicopter (Stirling et al. 1989). The drug Zoletil (Virbac Laboratories, Carros, France) was administered in a solution of 200 mg/mL at a dosage of 5–10 mg/kg of body mass. Blood samples were collected from the femoral vein into evacuated,

Address correspondence to B.M. Jenssen, Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway. Telephone: 47 7359 6267. Fax: 47 7359 1309. E-mail: bmj@bio.ntnu.no

Funding was provided by the Basic Pollution Research Program at the Norwegian University of Science and Technology (Norwegian Research Council Project 110686/420), and the Norwegian Research Council Projects 110750/720 and 125693/720.

Received 21 February 2002; accepted 21 November 2002.

heparinized containers and stored cool and dark until centrifugation (3,000 rpm for 10 min) within 8 hr of collection. Plasma was pipetted off and stored at –20°C until analysis. A vestigial premolar tooth was extracted from all bears for age determination (Calvert and Ramsay 1998).

Presence and age of cubs, and biologic parameters such as body length and axial girth were recorded. Body measurements were not recorded for one bear. Body mass was estimated using a morphometric equation (Derocher and Wiig 2002).

All capture dates were transformed to Julian dates (day 1–365), and age was calculated to the nearest month assuming that all bears were born on January 1st.

For the investigation of hormone and PCB concentrations, polar bears were grouped into three reproductive classes based on the presence and age of cubs. Females with 2-year-old offspring and single females were classified as single females (n = 32), because all these females enter estrus in spring and are thus available for mating (Wiig 1998). Because 2-year-old offspring receive very little milk (Polischuk et al. 1995), and the fat content in milk declines as the cubs grow older (Derocher et al. 1993), the transfer of PCBs to 2-year-olds through milk is presumed to be negligible. Females with nursing cubs less than 12 months of age were classed as females with cubs (n = 36), and those with dependent offspring 12–24 months of age were classed as females with yearlings (n = 18). When no statistical differences were found between the two groups "females with cubs" and "females with yearlings," these groups were pooled to form "females with offspring."

The Norwegian Experimental Animal Committee approved all capture and handling methods used.

PCB analyses. All polar bear plasma samples in the project were analyzed for OCs at the Environmental Toxicology Laboratory, Norwegian School of Veterinary Science/National Veterinary Institute, Oslo, Norway, from 1998 to 2000. The laboratory is accredited by Norwegian Accreditation as a testing laboratory for OCs according to the requirements of NS-EN45001 and ISO/IEC Guide 25. Briefly, plasma samples (approximately 8 g) were extracted with cyclohexane and acetone and cleaned up with ultra-pure sulfuric acid (Andersen et al. 2001). The whole extracts were used to determine percent extractable fat gravimetrically. Aliquots of the final extract were injected on gas chromatograph-electron capture detection (GC-ECD) instruments equipped with two capillary columns (SPB-5 and SPB-1701, 60 m, 0.25 mm ID and 0.25 µm film layer; Supelco, Inc., Bellefonte, PA, USA). Quantification was performed using PCB 29, 112, and 207 as internal standards in each sample. Details on analytic procedures, equipment for chromatographic separation, detection, and calculation are given in Andersen et al. (2001). Standard procedures were used to ensure adequate quality assurance and control, and the precision, linearity, and sensitivity of the analyses were within the laboratory's accredited requirements. A blank sample was included in each batch to test for interference. Reproducibility was continuously tested by analyzing the PCB levels in the laboratory's own reference sample (seal blubber). The mean percent of the true value and its coefficient of variation (CV) was 103 and 11% (n = 22). Recoveries and CV of all PCB congeners in spiked blood samples varied from 72 to 116% and 6.3 to 10.0%, respectively (n = 83). Detection limits for individual PCB congeners ranged from 0.002 to 0.02 ng/g wet weight. In all samples, the following 34 PCB congeners were determined (IUPAC numbers; Safe 1994): 28, 31, 47, 52, 56, 66, 74, 87, 99, 101, 105, 110, 114, 118, 128, 136, 137, 138, 141, 149, 151, 153, 156, 157, 170, 180, 183, 187, 189, 194, 196, 199, 206, and 209. Sixteen PCB congeners that could be quantified in all samples were selected for this study. These congeners were PCB 47, 99, 118, 128, 137, 138, 153, 156, 157, 170, 180, 183, 187, 189, 194, and 206. PCB congener concentrations were expressed on a lipid weight basis (ng/g lw) calculated from PCB concentrations in nanograms per gram wet weight and percentage of fat in the analyzed plasma.

Hormone analysis. Plasma concentrations of the steroid hormones P_4 and E_2 and the glucocorticoid cortisol were analyzed at the Hormone Laboratory at the Norwegian School of Veterinary Science using radioimmunoassay (RIA).

For the analysis of P_4, no modifications for polar bear plasma were necessary. The concentrations were analyzed using a commercially available kit (Spectria Progesterone ^{125}I Coated Tube Radioimmunoassay; Orion Diagnostica, Espoo, Finland). The kit detection limit was 0.3 nmol/L, corresponding to three standard deviations from the zero sample. The interassay coefficient of variation (CV) for control samples with low (1.39 nmol/L), medium (14.8 nmol/L), and high (44.1 nmol/L) concentrations of P_4 were 8.2, 6.3, and 5.3%, respectively.

E_2 concentrations were analyzed using a commercial kit (Coat-a-Count, Estradiol, Diagnostic Products Corporation, Los Angeles, CA, USA). The detection limit was 8.0 pg/mL, corresponding to 95% binding of the labeled hormone, assuming that basic procedures were followed. The E_2 concentration was low in the samples, so both the standard curve and the samples were modified to bring the concentrations within the detection limit. The standards were diluted with 0.02 M borate buffer, pH 8.5, to concentrations ranging from 10 to 600 pg/mL. Polar bear plasma samples were up-concentrated to bring E_2 concentrations within the linear range of the modified standard curve. We extracted 300 µL plasma from each sample with newly distilled diethyl ether, evaporated it to dryness, and added it to 150 µL borate buffer (0.02 M, pH 8.5), thus doubling the concentrations of E_2 in the plasma samples. Extraction recovery was 79.5 ± 4.1%, determined from a single recovery test per run, spiked with known amounts of the hormone. All concentrations were adjusted according to the recovery sample. The interassay CV for control samples with low (49.9 pg/mL), medium (83.1 pg/mL), and high (204.1 pg/mL) concentrations of E_2 were 6.4, 10.1, and 6.2%, respectively. Because of limited plasma volume, interassay CV control samples could not be made from polar bear plasma, and analyses were not run in parallel. The E_2 concentrations were therefore rounded to the nearest picogram per milliliter. E_2 analyses were not performed on five bears because of lack of plasma. Quantification of radiation when using RIA was performed using a 1470 Wizard Automatic Gamma counter (Wallac Oy, Turku, Finland).

Plasma cortisol was measured based on the methods described by Simensen et al. (1978) and was modified for polar bear plasma. Ten-microliter plasma samples were heated in 500 µL phosphate-buffered saline (PBS) with 0.1% gelatin for 20 min to remove effects from plasma proteins. Validation showed that serial dilution of heated polar bear plasma with high cortisol concentration produced a dose–response curve parallel to the standard curve. The range of the standard curve was 3.1–50 ng/mL, and the detection limit was 2 ng/mL, corresponding to 95% binding of the labeled hormone. Samples were diluted when concentrations were outside the range of the standard curve. After cooling, samples were extracted with 1 × 3 mL dimethylchloride, followed by evaporation to dryness under a gentle stream of nitrogen. Recovery was 79.9 ± 2.3%, determined from a single recovery test sample per run, spiked with known amounts of the hormone. All concentrations were adjusted according to the recovery sample. The dry extract was resolved in 1,200 µL PBS with 0.1% gelatin overnight. Two 500-µL samples were then pipetted off and run in parallel. After adding antiserum (No. F3-314; Endocrine Science Products, Tarzana, CA, USA) and ^{3}H-labeled cortisol mixture, the parallels were incubated for 1 hr at room temperature, then overnight at 4°C before centrifugation at 4°C with ice-cold charcoal–dextran suspension (15 min at 4,000 rpm). Scintillation liquid was added to the supernatant before counting in a Tri-carb 2100 TR

Table 1. Concentrations (nanomoles per liter) of P_4, E_2, and cortisol in plasma samples of three reproductive groups of female polar bears (*U. maritimus*) from Svalbard.

		P_4			E_2			Cortisol		
Reproductive class	Sample size	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% CI	Range
Single	32	2.8[a]	2.1–3.8	0.9–10.1	0.03[a]	0.03–0.04	0.01–0.07	391.3[a]	314.3–486.9	72.0–1301.8
With cubs	36	1.3[b]	1.2–1.5	0.7–2.2	0.03[a]	0.03–0.03	0.02–0.06	440.9[a]	360.62–538.9	150.6–1280.7
With yearlings	18	1.1[b]	0.9–1.4	0.5–1.9	0.03[a]	0.02–0.03	0.01–0.06	342.3[a]	229.7–509.9	39.7–807.8

Values are presented as means, range of 95% confidence interval (CI), and range of the measurements.
[a,b]Different superscripts indicate significant differences between groups.

Table 2. Concentrations (nanograms per gram lipid) of ΣPCB, the congener PCB-118 in plasma samples of three reproductive groups of female polar bears (*U. maritimus*) from Svalbard.

		ΣPCB (ng/g lipid)			PCB 118 (ng/g lipid)		
Reproductive class	Sample size	Mean	95% CI	Range	Mean	95% CI	Range
Single	32	9,740[a]	7,620–11,859	2,014–26,348	25[a]	21–30	8–62
With cubs	36	8,919[a]	7,746–10,091	2,082–18,210	21[a]	18–23	8–44
With yearlings	18	5,895[b]	4,128–7,653	1,392–15,172	25[a]	19–30	7–52

Values are presented as means, range of 95% CI, and range of the measurements.
[a,b]Different superscripts indicate significant differences between groups.

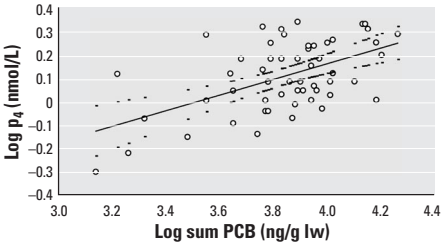

Figure 1. In female polar bears with offspring, the plasma P_4 concentration was positively related to plasma ΣPCBs concentrations. The regression line (solid) and 95% CIs (dotted) are indicated (r^2 = 0.27).

Liquid Scintillation Analyzer (Packard, Downers Grove, IL, USA).

The interassay CV for control samples with low (4.85 ng/mL), medium (16.9 ng/mL), and high (41.5 ng/mL) concentrations of cortisol were 7.2, 6.5, and 7.0%, respectively.

Statistical analyses. All analyses and calculations were conducted using a statistical package (SPSS 10.0, SPSS Inc., Chicago, IL, USA). The level of statistical significance was defined at $p \leq 0.05$. To obtain normal distribution of investigated parameters, age (years, months), ΣPCBs (sum of all analyzed polychlorinated biphenyl congeners) (ng/g lw), PCB-118 (ng/g lw), E_2 (pg/mL), P_4 (nmol/L), and cortisol (ng/mL), were $\log_{10}$-transformed before analysis, and converted to nanomoles per liter after analysis (E_2 and cortisol). ∑PCBs refer to the sum of all congeners except PCB-118, i.e., 15 congeners. PCB-118 was not correlated with ΣPCBs for females with cubs (n = 36, r = –0.24, p = 0.15), females with yearlings (n = 18, r = 0.46, p = 0.06), or single females (n = 32, r = –0.221, p = 0.225) and was therefore investigated separately in the statistical analyses. The reason for this may be because PCB-118 has a rather low biomagnification factor from seal to polar bear (Letcher et al. 1996), indicating that it is metabolized relatively quickly by the polar bear. Geometric means were calculated from log-transformed values. Means were compared using one-way analysis of variance (ANOVA). For *post hoc* multiple comparison of groups, Dunnett's T3 test for groups with unequal variance was used. In addition, data were subjected to correlation analysis using the parametric Pearson correlation. Multiple regression analysis (backward selection method) was used to study extrinsic and intrinsic independent variables associated with variation in hormone concentrations. The independent variables were capture date (Julian date), age (years, months), body mass (kilograms), ΣPCBs (nanograms per gram lipid weight), and PCB-118 (nanograms per gram lipid weight). Effects on plasma P_4 concentrations due to stress of handling were also examined, because stress-related cortisol production may lead to higher blood levels of adrenal P_4 (Harlow et al. 1990; Plotka et al. 1983).

Results

The plasma P_4 concentration was significantly higher in single females than in females with cubs and in females with yearlings ($F_{2.83}$ = 21.7, p < 0.001; Table 1). There was no difference in P_4 concentrations between females with cubs and females with yearlings (Dunnett's T3, p = 0.3; Table 2). The plasma E_2 concentrations did not differ between the reproductive groups ($F_{2.78}$ = 2.5, p = 0.09; Table 1), and ranges were similar for all reproductive groups.

E_2 and P_4 concentrations of females with cubs and females with yearlings did not differ, presumably because all females with nursing offspring (cubs and yearlings) are in lactational anestrus. The two groups were thus pooled into one group (n = 54) to study relationships between the independent factors and P_4. In females with offspring, the P_4 concentrations were related only to logΣPCBs (estimate = 0.326, E = 0.073, $F_{1.51}$ = 20.15, p < 0.001, r^2_{adj} = 0.269; Figure 1) and not to age, body mass, capture date, PCB-118, or cortisol (F < 2.71, p > 0.21).

Multiple regression analysis showed that P_4 concentrations in the single females were related only to the independent factor capture date (estimate = 0.01202, SE = 0.003, $F_{1.31}$ = 12,61, p = 0.001, r^2_{adj} = 0.273) and not to the factors age, body mass, ΣPCB, PCB-118, or cortisol (F < 2.71, p > 0.16). Thus, P_4 concentrations for single females increased throughout the capture period (Figure 2).

The E_2 concentrations were not significantly related to any of the predictors reproductive group, age, body mass, capture date, cortisol, ΣPCBs, or PCB-118 (F < 2.71, p > 0.21). However, there was a borderline negative relationship between E_2 and PCB-118 in females with offspring (n = 54, coefficient = –0.227, p = 0.062, r^2_{adj} = 0.05). Plasma concentrations of cortisol did not differ significantly between the reproductive groups ($F_{2.83}$ = 0.95, p = 0.4; Table 1).

ΣPCBs concentrations differed between the three reproductive groups (Table 2). Females with yearlings had lower ΣPCBs concentrations than single females and females with cubs (ANOVA, $F_{2.83}$ = 6.5, p = 0.002). ΣPCBs concentrations did not differ between females with cubs and single females (Dunnett's T3, p = 1.0). PCB-118 did not differ between the reproductive classes (ANOVA, $F_{2.83}$ = 1.2, p = 0.3). Plasma fat did not differ between the reproductive classes (ANOVA, $F_{2.83}$ = 0.7, p = 0.5).

Females with cubs were significantly younger than females with yearlings (ANOVA, $F_{2.83}$ = 5.4, p = 0.006), but not younger than single females (Dunnett's T3, p = 0.1; Table 3). The body mass of females with cubs was significantly lower than that of single females (ANOVA, $F_{2.82}$ = 4.9, p = 0.01), whereas the body mass of females with yearlings did not differ from that of either females with cubs or single females (Dunnett's T3, p = 0.4 and 0.6, respectively).

Discussion

In female polar bears with offspring, the plasma concentration of ΣPCBs explained 27% of the variation in the P_4 levels. To our knowledge, a positive relationship between ΣPCBs and P_4 in lactating female mammals has not previously been documented. Previously, a study on pregnant mink documented that serum P_4 concentrations were significantly increased by experimental exposure to PCB-153 (Patnode and Curtis 1994). Furthermore, an *in vitro* study has documented significantly increased

secretion of P_4 in granulosa cells from porcine ovaries after exposure to PCB-153 (Wojtowicz et al. 2000). Thus, although the relationship between ΣPCBs and P_4 reported herein in female polar bears does not prove a causal exposure–effect relationship per se, the result represents an indication that PCBs may affect levels of P_4 in polar bear females.

P_4 is synthesized in the maturing follicles as a step in the E_2 synthesis. Stimulation of the enzymes preceding P_4 or inhibition of enzymes following P_4 in the synthesis may lead to an increase in P_4 concentrations. In common seals, P_4 metabolism was significantly decreased with increasing liver PCB concentration (Troisi and Mason 2000). An inhibition of the enzymes transforming P_4 to E_2 has been suggested as one explanation for the increased P_4 secretion and decreased testosterone and E_2 secretion by granulosa cells after exposure to PCB-153 (Wojtowicz et al. 2000). PCB-153 is one of the most commonly detected PCB congeners in biologic tissues and is the dominant PCB congener in polar bears (Bernhoft et al. 1997; Norheim et al. 1992; Norstrom et al. 1998).

The exact mechanisms for the many observed reproductive effects of PCBs are poorly understood. One effect of P_4 is to exert negative feedback on secretion of gonadotropin-releasing hormone from the hypothalamus, thus inhibiting secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary (Richards 1986). PCB-induced elevation of P_4 may thus affect FSH/LH-stimulated maturation of follicles in single females and prevent normal ovulation from taking place. Previously, disruption of hormonal feedback mechanisms caused by increased P_4 concentrations during estrus has been demonstrated in rabbits (Chang 1967).

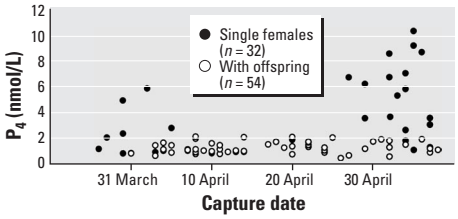

Figure 2. In single female polar bears, P_4 concentrations increased throughout the capture period (March–May 1995–1998), whereas no such relationship was observed in females with offspring.

Furthermore, slightly elevated P_4 concentrations in heifers at the time of artificial insemination led to significantly lower fertility (Waldman et al. 2001). Functionally, it is therefore possible that PCB-induced elevation of P_4 concentrations in polar bears may affect reproductive success.

The higher P_4 concentrations in single females than in females with cubs and females with yearlings can be explained by physiologic differences due to reproductive status. Females with nursing offspring are anestrus and will not ovulate and form a P_4-secreting corpus luteum. P_4 concentrations for these two reproductive groups are therefore low. On the contrary, the higher P_4 concentrations in single females are due to the formation of a P_4-producing corpus luteum after ovulation (Tsubota et al. 1994; Wimsatt 1963). This also explains the increasing concentrations of P_4 with capture date.

The P_4 concentrations reported in the single female polar bears herein are consistent with those previously reported in solitary females from the Canadian Arctic during February and March (Derocher et al. 1992; Ramsay and Stirling 1988). Because levels of PCBs are lower in Canadian compared with Svalbard polar bears (Norstrom et al. 1998), the assumption of a positive relationship between PCB burden and P_4 should result in higher P_4 concentrations in solitary polar bears from Svalbard compared with those from the Canadian Arctic. The apparent lack of such a finding may be because P_4 levels in single female bears vary considerably over a short time during the spring (Figure 2), depending on when samples are taken relative to their ovulation. The resulting large variation may override possible effects caused by PCB exposure, rendering it impossible to evaluate the effect of PCB on P_4 in single female polar bears. To study possible effects of PCB on P_4 in single polar bear females, the timing of the sampling should be standardized in relation to their ovulation.

Reijnders (1986) has reported a lower E_2 concentration in plasma of common seals fed PCB-contaminated fish than in the control group, and several studies have shown that different PCB congeners and their metabolites may exert both estrogenic and antiestrogenic effects through a wide range of mechanisms (Battershill 1994; Connor et al. 1997; Danzo 1998; Dechaud et al. 1999; Kester et al. 2000; Moore et al. 1997; Nesaretnam et al. 1996; Wojtowicz et al. 2000). In this study, no relationships between E_2 and the investigated factors (age, body mass, capture date, cortisol, ΣPCBs, and PCB-118) were found. However, a borderline negative relationship between E_2 and PCB-118 (n = 54, p = 0.062) was found in females with offspring. This indicates that further studies are required to assess the possible relationship between PCBs and estrogen in polar bears.

A severalfold variation in the E_2 concentration was observed within each of the three reproductive groups, but there were no differences in mean E_2 concentrations between the groups. Because the rate of E_2 secretion from the maturing follicle increases before ovulation (Senger 1997), single females were expected to have higher E_2 concentrations than anestrous (lactating) females in spring. It may therefore be speculated that the E_2 concentrations in the single females may be suppressed because of PCB exposure. However, no evidence can be given to support such a speculation. On the contrary, large variation in E_2 concentrations has been reported in female black bears (*Ursus americanus*) during the periimplantation period (Hellgren et al. 1991; Tsubota et al. 1998). It is possible that similar natural large variations were present in the single polar bears around the time of ovulation, and that this was the reason why their mean E_2 concentration did not differ from that of the other reproductive groups.

The E_2 concentrations reported herein (0.0–0.07 nmol/L) are low compared with the concentrations reported for polar bears near Churchill, Manitoba, Canada, and on the Beaufort Sea (approximately 0.07–0.09 nmol/L) (Palmer et al. 1988). Similar methods (RIA) were used for determination of hormone concentrations in the two studies. Because levels of PCBs are higher in polar bears from Svalbard compared with Canadian polar bears (Norstrom et al. 1998), the lower E_2 concentration in Svalbard bears is in accordance with the prediction that PCB lowers the levels of circulating estrogen. However, it is also possible that the interpopulation difference in E_2 is caused by a wide range of confounding factors, such as the age structure of the animals in the two studies, their reproductive status relative to ovulation, and their nutritional status. Thus, further investigations are needed to explain the apparent interpopulation

Table 3. Age, body mass, and plasma fat content in the three reproductive groups of female polar bears (*U. maritimus*) from Svalbard.

		Age (years:months)			Body mass (kg)			Plasma fat (%)		
Reproductive class	Sample size	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% CI	Range
Single	32	11:7[a,b]	9:6–13:8	5:2–26:4	190[a]	182–199	133–234	1.2[a]	1.1–1.3	0.6–1.8
With cubs	36	8:10[a]	7:8–10:1	4:4–21:4	174[b]	168–180	136–214	1.2[a]	1.1–1.3	0.8–1.8
With yearlings	18	12:7[b]	10:6–14:10	5:4–20:4	183[a,b]	172–194	152–234	1.3[a]	1.1–1.4	0.5–1.7

Values are presented as means, range of 95% CI, and range of the measurements
[a,b]Different superscripts indicate significant differences between groups.

difference in E_2 between female polar bears from Svalbard and Canada.

The cortisol concentrations in this study showed large variations between individuals, and the cortisol levels were higher than those reported for female black bears caught in foot snares during spring (March–May) (Harlow et al. 1990). The concentrations may be elevated as a consequence of immobilization and handling, but there seemed to be no differential effect of handling between reproductive groups. Because there was no relationship between cortisol and P_4 concentrations, we conclude that P_4 concentrations in the bears were not affected by elevated cortisol. The same conclusion was reached by Harlow and co-workers (1990) when they compared the stressful snare trapping of brown bears (*Ursus arctos*) in summer to winter-den sampling.

The plasma PCB concentrations in the female polar bears at Svalbard during spring in 1995–1998 were similar to those reported in blood samples and adipose tissue of female polar bears at Svalbard during spring in 1990–1994 (Bernhoft et al. 1997; Norstrom et al. 1998). ΣPCBs did not differ between females with cubs of the year and single females, but was significantly lower in females with yearlings. Because the body mass of females with yearlings did not differ from that of the other reproductive groups, prolonged transfer of OCs from mothers to cubs through the lipid-rich milk may be the major cause of the lower levels in the females with yearlings (Addison and Brodie 1977; Oehme et al. 1995; Polischuk 1999; Polischuk et al. 1995). Reproductive history is thus probably one of the major factors affecting accumulation of PCBs in female polar bears, and females that rear cubs successfully may reduce their body burden of PCBs by excreting contaminants through milk at a greater rate than intake. The comparable PCB concentrations reported herein in the lean lactating females with cubs and the heavier, nonlactating single females may reflect both the role of lactation as an excretory route for PCBs and the significance of dilution of PCBs in the storage lipids of nonlactating females. PCB-118 did not correlate with the congeners that constitute ΣPCB, and the concentration of this specific congener did not differ between the three reproductive groups. This suggests that PCB-118 has different kinetics in polar bears than the other congeners; indeed, this congener seem to be relatively readily metabolized by polar bears (Letcher et al. 1996).

The relative scarcity of female polar bears ≥ 16 years of age with cubs of the year in the Svalbard population compared with the other polar bear populations has been interpreted as a sign that reproduction in the Svalbard polar bear population may be impaired (Derocher et al. 2003). Even though it is functionally possible that a PCB-induced elevation of P_4 concentrations in polar bears may reduce their fertility, it is not possible to make a causal link between the low reproduction rate in the Svalbard population of polar bears (Derocher et al. 2003) and PCBs.

Conclusions

In female polar bears with offspring, concentrations of ΣPCBs explained 27% of the variation in the P_4 concentrations. PCBs causing either increased synthesis or decreased transformation of P_4 may explain the observed positive correlation between P_4 and ΣPCBs. Although this relationship is not evidence per se of a direct cause–effect association, the results may represent an indication that PCBs may affect levels of P_4 in polar bear females. There is a clear need to further assess the hormone economy and population health of polar bears at Svalbard.

References

Addison RF, Brodie PF. 1977. Organochlorine residues in maternal blubber, milk, and pup blubber from grey seals (*Halichoerus grypus*) from Sable Island, Nova Scotia. J Fish Res Board Can 34:937–941.

Andersen M, Lie E, Derocher AE, Belikov SE, Bernhoft A, Boltunov AN, et al. 2001. Geographic variation of PCB congeners in polar bears (*Ursus maritimus*) from Svalbard east to the Chukchi Sea. Polar Biol 24:231–238.

Backlin BM, Persson E, Jones CJP, Dantzer V. 1998. Polychlorinated biphenyl (PCB) exposure produces placental vascular and trophoblastic lesions in the mink (*Mustela vison*): a light and electron microscopic study. APMIS 106:785–799.

Battershill JM. 1994. Review of the safety assessment of polychlorinated-biphenyls (PCBs) with particular reference to reproductive toxicity. Hum Exp Toxicol 13:581–597.

Bergman A, Olsson M. 1985. Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? Finnish Game Res 44:47–62.

Bernhoft A, Wiig O, Skaare JU. 1997. Organochlorines in polar bears (*Ursus maritimus*) at Svalbard. Environ Pollut 95:159–175.

Calvert W, Ramsay MA. 1998. Evaluation of age determination of polar bears by counts of cementum growth layer groups. Ursus 10:449–453.

Chang MC. 1967. Effects of progesterone and related compounds on fertilisation, transportation, and development of rabbit eggs. Endocrinology 84:356–361.

Colborn T, Saal FSV, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384.

Connor K, Ramamoorthy K, Moore M, Mustain M, Chen I, Safe S, et al. 1997. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: structure-activity relationships. Toxicol Appl Pharmacol 145:111–123.

Danzo BJ. 1998. The effects of environmental hormones on reproduction. Cell Mol Life Sci 54:1249–1264.

Dechaud H, Ravard C, Claustrat F, De la Perriere AB, Pugeat M. 1999. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). Steroids 64:328–334.

Derocher AE, Andriashek D, Arnould JPY. 1993. Aspects of milk composition and lactation in polar bears. Can J Zool 71:561–567.

Derocher AE, Stirling I, Andriashek D. 1992. Pregnancy rates and serum progesterone levels of polar bears in western Hudson Bay. Can J Zool 70:561–566.

Derocher AE, Wiig Ø. 2002. Postnatal growth in body length and mass of polar bears (*Ursus maritumus*) at Svalbard. J Zool Lond 256:343–349.

Derocher AE, Wolkers H, Colborn T, Schlabach M, Larsen TS, Wiig O. 2003. Contaminants in Svalbard polar bear samples archived since 1967 and possible population level effects. Sci Total Environ 301:163–174.

Harlow HJ, Beck TDI, Walters LM, Greenhouse SS. 1990. Seasonal serum glucose, progesterone and cortisol levels of black bears (*Ursus americanus*). Can J Zool 68:183–187.

Helle E, Ollsson M, Jensen S. 1976. DDT and PCB levels and reproduction in ringed seals from the Bothnian Bay. Ambio 5:188–189.

Hellgren EC, Vaughen MR, Gwasdauskas FC, Williams B, Scanlon PF, Kirkpatrick RL. 1991. Endocrine and electrophoretic profiles during pregnancy and non-pregnancy in captive female black bears. Can J Zool 69:892–898.

Kester MHA, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, et al. 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 141:1897–1900.

Kihlstrom JE, Olsson M, Jensen S, Johansson A, Ahlbom J, Bergman A. 1992. Effects of PCB and different fractions of PCB on the reproduction of the mink (*Mustela vison*). Ambio 21:563–569.

Letcher RJ, Norstrom RJ, Lin S, Ramsay MA, Bandiera SM. 1996. Immunoquantitation and microsomal monooxygenase activities of hepatic cytochromes P4501A and p4502B and chlorinated hydrocarbon contaminant levels in polar bear (*Ursus maritimus*). Toxicol Appl Pharmacol 137:127–140.

Lønø O. 1970. The polar bear (*Ursus maritimus* Phipps) in the Svalbard area. Norsk Polarinstitutt Skrifter 149:1–103.

Moore M, Mustain M, Daniel K, Chen I, Safe S, Zacharewski T, et al. 1997. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicol Appl Pharmacol 142:160–168.

Muir DCG, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ. 1992. Arctic marine ecosystem contamination. Sci Total Environ 122:75–134.

Nesaretnam K, Corcoran D, Dils RR, Darbre P. 1996. 3,4,3′,4′-Tetrachlorobiphenyl acts as an estrogen *in vitro* and *in vivo*. Mol Endocrinol 10:923–936.

Norheim G, Skaare J, Wiig Ø. 1992. Some heavy metals, essential elements, and chlorinated hydrocarbons in polar bear (*Ursus maritimus*) at Svalbard. Environ Pollut 77:51–57.

Norstrom RJ, Belikov SE, Born EW, Garner GW, Malone B, Olpinski S, et al. 1998. Chlorinated hydrocarbon contaminants in polar bears from eastern Russia, North America, Greenland, and Svalbard: biomonitoring of Arctic pollution. Arch Environ Contam Toxicol 35:354–367.

Norstrom RJ, Muir DCG. 1994. Chlorinated hydrocarbon contaminants in arctic marine mammals. Sci Total Environ 154:107–128.

Oehme M. 1991. Dispersion and transport paths of toxic persistent organochlorines to the Arctic—levels and consequences. Sci Total Environ 106:43–53.

Oehme M, Biseth A, Schlabach M, Wiig O. 1995. Concentrations of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and non-ortho substituted biphenyls in polar bear milk from Svalbard (Norway). Environ Pollut 90:401–407.

Palmer SS, Nelson RA, Ramsay MA, Stirling I, Bahr JM. 1988. Annual changes in serum sex steroids in male and female black (*Ursus americanus*) and polar (*Ursus maritimus*) bears. Biol Reprod 38:1044–1050.

Patnode KA, Curtis LR. 1994. 2,2′,4,4′,5,5′-Hexachlorobiphenyl and 3,3′,4,4′,5,5′- hexachlorobiphenyl alteration of uterine progesterone and estrogen-receptors coincides with embryotoxicity in mink (*Mustela vision*). Toxicol Appl Pharmacol 127:9–18.

Plotka ED, Seal US, Verme LJ, Ozoga JJ. 1983. The adrenal gland in white-tailed deer: a significant source of progesterone. J Wildl Manage 47:38–44.

Polischuk SC. 1999. Organochlorine dynamics in free-ranging polar bears and their cubs [PhD Thesis]. Saskatoon, Saskatchewan, Canada:University of Saskatchewan.

Polischuk SC, Letcher RJ, Norstrom RJ, Ramsay MA. 1995. Preliminary-results of fasting on the kinetics of organochlorines in polar bears (*Ursus maritimus*). Sci Total Environ 161:465–472.

Ramsay MA, Stirling I. 1988. Reproductive biology of female polar bears (*Ursus maritimus*). J Zool Lond 214:601–634.

Richards JAS. 1986. Molecular loci for potential drug toxicity in ovaries. Environ Health Perspect 70:159–161.

Safe SH. 1994. Polychlorinated-biphenyls (PCBs)—environmental-impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149.

Senger P. 1997. Pathways to Pregnancy and Parturition. Pullman, WA:Current Conceptions, Inc., Washington State University and Technology Park.

Skaare JU, Bernhoft A, Derocher A, Gabrielsen GW, Goksoyr A,

Henriksen E, et al. 2000. Organochlorines in top predators at Svalbard—occurrence, levels and effects. Toxicol Lett 112:103–109.

Smith TG. 1980. Polar bear predation of ringed and bearded seals in the land-fast sea ice habitat. Can J Zool 58:2201–2209.

Stirling I, McEwan EH. 1975. The calorific value of whole ringed seals (*Phoca hispida*) in relation to polar bear (*Ursus maritimus*) ecology and hunting behaviour. Can J Zool 53:1021–1027.

Stirling I, Spencer C, Andriashek D. 1989. Immobilisation of polar bears (*Ursus maritimus*) with Telazol in the Canadian arctic. J Wildl Dis 25:159–168.

Troisi GM, Mason CF. 2000. PCB-associated alteration of hepatic steroid metabolism in harbor seals (*Phoca vitulina*). J Toxicol Environ Health 61A:649–655.

Tsubota T, Howell-Skalla L, Boone WR, Garshelis DL, Bahr JM. 1998. Serum progesterone, oestradiol, luteinizing hormone and prolactin profiles in the female black bear (*Ursus americanus*). Anim Reprod Sci 53:107–118.

Tsubota T, Nitta H, Osawa Y, Kita I, Tiba T, Bahr JM. 1994. Immunolocalization of steroidogenic enzymes p450scc, 3-beta-hsd, p450c17 and p450arom in the corpus-luteum of the Hokkaido brown bear (*Ursus arctos* Yesoensis) in relation to delayed implantation. J Reprod Fertil 101:557–561.

Waldman A, Reksen O, Landsverk K, Kommisrud E, Dahl E, Refsdal AO, et al. 2001. Progesterone concentrations in milk fat at first insemination—effects on non-return and repeat breeding. Anim Reprod Sci 65:33–41.

Wiig Ø. 1998. Survival and reproductive rates for polar bears at Svalbard. Ursus 10:25–32.

Wiig Ø, Derocher AE, Cronin NM, Skaare JU. 1998. Female pseudohermaphrodite polar bears at Svalbard. J Wildl Dis 34:792–796.

Wimsatt WA. 1963. Delayed implantation in the *Ursidae*, with particular reference to the black bear (*Ursus americanus* Pallas). In: Delayed Implantation (Enders AC, ed). Chicago:The University of Chicago Press, 49–76.

Wojtowicz AK, Gregoraszczuk EL, Lyche JL, Ropstad E. 2000. Time dependent and cell-specific action of polychlorinated biphenyls (PCB 153 and PCB 126) on steroid secretion by porcine theca and granulosa cells in mono- and co-culture. J Physiol Pharmacol 51:555–568.