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Menstrual cycle characteristics in European and Inuit women exposed to persistent organochlorine pollutants

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BACKGROUND: Previous inconsistent results suggest that menstrual cycles may be disturbed by exposure to polychlorinated biphenyls (PCBs) and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (DDE). METHODS: Information on menstrual cycle characteristics were obtained by questionnaires, and PCB and DDE were measured in serum samples from a total of 1494 women from Greenland, Swedish fishermen's wives, and inhabitants of Warsaw in Poland and Kharkiv in Ukraine. RESULTS: No consistent effects of PCB and DDE exposure on menstrual cycle characteristics were observed across populations. Within populations, we observed increased risks of short cycles (≤24 days) among Swedish fishermen's wives exposed to high levels of PCB [odds ratio (OR) 2.5, confidence interval (CI) 1.2–5.1], and increased risk of long cycles (≥32 days) among Polish women exposed to high levels of DDE (OR 3.1, CI 1.1–8.6). However, in Greenland it seemed that high levels of PCB or DDE were protective against long menstrual cycles (OR 0.7 CI 0.5–0.96 and OR 0.7 CI 0.5–0.99, respectively). CONCLUSIONS: It is unlikely that exposure to PCB and DDE is a main cause of menstrual disturbances. Genetic differences or dietary factors may be involved in the non-homogenous associations of organochlorine exposure and menstrual cycle between countries.

Keywords: contaminants; endocrine disruptors; irregularity; menses

Introduction

Disruption of the menstrual cycle is associated with impaired fecundability (Jensen *et al*., 1999), and therefore an important health problem for women desiring to conceive.

Several factors have been associated with menstrual cycle disturbances including: age, body mass index (BMI), smoking (Rowland *et al*., 2002), extreme exercise (Chen and Brzyski, 1999), work stress (Hatch *et al*., 1999), organic solvents and other chemical compounds (Cho *et al*., 2001).

Some of the xenobiotics found in relatively high concentrations in blood samples from humans are organochlorines, and especially the persistent organochlorines such as polychlorinated biphenyls (PCBs) and metabolites of dichlorodiphenyltrichloroethane (DDT). In this study, 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) was used as a biomarker for PCB exposure. It has been shown that PCB-153 correlates very well with total PCB concentration in plasma and serum from Swedish subjects and Inuits from Greenland. (Grimvall *et al*., 1997; Glynn *et al*., 2000). The metabolite, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (DDE) reflects the long-term exposure to DDT due to a longer half-live and has been used as a biomarker of exposure to the insecticide DDT (ATSDR, 2002). Both biomarkers occur in such levels that a large number of analyses can be performed with good precision and accuracy and to a reasonable cost.

In vitro studies indicate that PCB and DDE may affect the endocrine system, exhibiting (anti)estrogenic and/or antiandrogenic effects (Kelce *et al*., 1995; Bonefeld-Jorgensen *et al*., 2001). In addition, *in vitro* experiments with porcine granulosa cells has demonstrated inhibited steroidogenesis after DDE and PCB exposure with reduced progesterone secretion as the most marked effect (Chedrese and Feyles, 2001; Wojtowicz *et al*., 2001). Progesterone and estradiol (E_2) are essential hormones controlling the menstrual cycle, and therefore it is plausible that chemicals disrupting their production/function may disturb menstrual cyclicity. *In vivo* studies in rats and monkeys indicate effects of organochlorines on hormone level and menstrual cycle (Jonsson *et al*., 1975; Barsotti *et al*., 1976; Brezner *et al*., 1984; Bryce *et al*., 2000). Also in humans studies indicate decreased progesterone and estrogen levels at higher DDE concentrations (Windham *et al*., 2005; Perry *et al*., 2006). Specifically, decreased E_2 concentration were found around the time when its peak is necessary for ovulation and decreased progesterone were

found around the time when rising progesterone are essential for early pregnancy maintenance (Perry *et al*., 2006). Thus both of these alterations may lead to reduced fecundability among humans.

The effects of organochlorine exposure on menstrual cycle characteristics in humans has been assessed in previous studies (Mendola *et al*., 1997; Yu *et al*., 2000; Axmon *et al*., 2004; Chen *et al*., 2005; Cooper *et al*., 2005; Ouyang *et al*., 2005; Windham *et al*., 2005; Chao *et al*., 2007), but findings are not consistent. Only few studies contain large populations and accurate exposure assessment of PCB and DDE (Cooper *et al*., 2005; Ouyang *et al*., 2005). Therefore the effects, if any, of PCB and DDE on the menstrual cycle are still not fully understood. In the present study we examine the association between measured PCB and DDE exposure and menstrual cycle length and irregularities among women collected for a European study of fertility in relation to organochlorine exposure (www.inuendo.dk), thereby representing one of the largest databases of European populations with measured PCB and DDE exposure so far (Jönsson *et al*., 2005).

Materials and Methods

Study populations

The target population was pregnant women who had antenatal care visits between June 2002 and May 2004, at local hospitals in 19 municipalities and settlements in Greenland, at a large central hospital in Warsaw, Poland and at three hospitals and eight antenatal clinics in Kharkiv, Ukraine. With few exceptions, the antenatal care programs cover all pregnant women in these localities. In addition, non-pregnant Swedish fishermen's wives were identified using a cohort of fishermen living at the west or east coast of Sweden originally created to study health effects related to PCB exposure. All eligible women received written and oral information on the main purpose of the study—fertility in relation to organochlorine exposure—before they decided if they wanted to participate. All women were asked to fill in a questionnaire and have a blood sample drawn. A more detailed description of the recruitment procedure and participation can be found in Toft *et al*. (2005). A standard questionnaire was developed in English and translated to the language of the respective study populations. The exact same questionnaire was used for the pregnancy-based populations, whereas the questionnaire for the Swedish fishermen's wives was slightly modified. A general criterion for eligibility was that the women should be $\geq$18 years of age. All together 2269 women were interviewed, and the participation rates were 90% among Inuits from Greenland, 68% in Warsaw, 26% in Kharkiv and 39% among the Swedish fishermen's wives. Some overlap between a previous study on menstrual cycle by Axmon *et al*. (2004) and the present study was found where 354 out of the 463 fishermen's wives also participated in the previous study. However, PCB-153 was only estimated for 41 of the women included in both the previous and the present study. The study was approved by the local ethical committees and all included women filled in an informed consent.

Exposure to PCB and DDE

Blood samples were drawn from a cubital vein into 10 ml vacuum tubes for serum collection without additives (Becton Dickinson, Moylan, France). After centrifugation the serum were transferred to ethanol rinsed brown glass bottles (Termometerfabrikken, Gothenburg, Sweden) and stored at -20°C until shipment on dry ice. After shipment to the Divison of Occupational and Environmental Medicine and Psychiatric Epidemiology in Lund, Sweden, the samples were kept at -80°C until analysis. The analysis procedure has been described in detail elsewhere (Jönsson *et al*., 2005). Briefly, the samples were processed applying solid-phase extraction using on-column degradation of the lipids and analyzed by gas chromatography–mass spectrometry. The analyses of PCB-153 and DDE were part of the Round Robin intercomparison program (Professor Hans Drexler, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany) with analysis of results found to be within the tolerance limits. Levels of detection, coefficients of variation and participation in quality control programs have been described in detail elsewhere (Jönsson *et al*., 2005). Serum lipid concentration was determined based on enzymatic determination of triglycerides and cholesterol. The lipid concentration in serum (gram per liter) was calculated by the following equation: total = 0.96 + 1.28 (triglycerides + cholesterols) (Rylander *et al*., 2006).

The Swedish fishermen's wives had the serum samples collected several years after the time period they were asked to refer to regarding menstrual cycle characteristics. We estimated the exposure at the relevant time period (the time just prior to the last planned pregnancy) using a previously validated model (Axmon and Rignell-Hydbom, 2006). The reduction of both PCB-153 and DDE due to breastfeeding was calculated as a 20% reduction for less than six months of breastfeeding and 30% for more than six months of breastfeeding. For PCB-153, a human biological half-life of 5 years was assumed during non-lactational periods and in addition a 3% yearly reduction in CB-153 in fatty fish from the Baltic Sea (the main exposure source for the fishermen's wives) from 1976 and onwards was included in the model. For DDE, the biological half-life was set to 8 years and the reduction in Baltic Sea fish was 20% between 1971 and 1981 and 9% yearly thereafter.

These estimated concentrations were used in all the presented results regarding the Swedish fishermen's wives.

Outcome

The participants were asked about their menstrual cycle characteristics in the time period when they tried to become pregnant using a validated questionnaire (Juul *et al*., 1999). Specifying that the woman should think about the period when she was trying to become pregnant, the following question was used to assess menstrual cycles among the pregnant women:

How long was it from the start of one menstrual bleeding to the start of the next bleeding?

- Number of days: ___ or
- Between ___ and ___ days
- No bleeding at all ☐
- I can't remember, don't know ☐

Women who reported a difference of 7 days or more in cycle lengths between months were defined to have irregular cycles. This measure could not be determined for the Swedish women who only reported the average cycle length prior to the latest planned pregnancy with a median of 19 years recall time. Short cycles was defined as 24 days or less, whereas long cycles was 32 days or more based on the distribution of cycle lengths and fertility in relation to cycle lengths in prospective studies (Kolstad *et al*., 1999; Small *et al*., 2006). Information on potential confounders (age, BMI, parity, smoking and drinking) was obtained with reference to the same time as information regarding menstrual cycle length, except for the Swedish women who reported current BMI. Furthermore, the women were asked about their age when they had their first menstrual periods.

Non respondents, excluded or missing data

Out of the 2269 interviews performed, 1881 provided data on menstrual cycle length. However, 11 of these were excluded due to average cycle length <10 (which are probably reporting errors), 141 were excluded due to menstrual cycles controlled by oral contraceptives, and 235 had missing information on PCB or DDE exposure, ending up with a final population of 1494 women.

The potential confounders did not differ between the excluded and included data, except for the excluded Ukrainians being slightly younger than the included (on average 1 year), and the excluded Swedes having a higher BMI (on average 2 kg/m^2).

Statistics

The associations between PCB and DDE exposure (log transformed) and menstrual cycle length were modeled by multiple regression models, controlling for the potential confounding effects of age (log transformed), BMI, parity, alcoholic beverages (drinks/week) and smoking (yes/no). When the predictor in a linear regression is log transformed, the interpretation of the effect estimate, the beta coefficient, is not straight forward. However, beta multiplied by ln (1.1) can be interpreted as the change in the average value of the outcome for every 10% increase in the predictor (Vittinghoff *et al*., 2005). Thus, the results from the linear regression models are presented after transformations using this formula. Restriction to women with minor deviations in cycle length (periods between 20 and 40 days) was made to evaluate strength of the observed findings.

Odds ratios (ORs) for short cycles and long cycles were estimated by logistic regression, using cycles with a length between 24 and 32 days as reference and controlling for the same potential confounders as listed above. For the logistic regressions, PCB-153 and DDE levels were trichotomized, at levels giving a reasonable number of subjects in each group, except for the Polish study group where all samples were classified as low PCB-153 (<50 ng/g lipid). In addition, logistic regression analysis with continuous PCB-153 and DDE (log transformed) were performed.

Results

The shortest cycle length was found among the women from Ukraine and also the Swedish fishermen's wives had relative short average cycle lengths, whereas longer average cycle lengths were found among the Polish and the Greenlandic women (Table I). Irregular cycles were most prevalent among the Greenlandic women, and less so among the women from Ukraine. Short cycles (≤24 days) were most often found among the women from Ukraine, but very seldom among women from Poland or Greenland, whereas long cycles (≥32 days) were found among almost 1/5 of the Polish women, but only among 3% of the Greenlandic women.

The distributions of the reported cycles lengths can be found in Fig. 1, indicating similar distributions, except for a slightly higher digit preference for 28 days in Sweden and Greenland.

The age at menarche differed between study populations, with the lowest age at menarche found among the girls from Greenland and the highest age among the Polish girls. Also the other covariates: age, BMI, parity, drinking and smoking varied between countries. Especially the Swedish fishermen's wives were older and had more previous children than the women from the other three populations. This may, however, be explained by the difference in sampling procedure. Moreover, the Swedish women had higher current BMI than the other women, which is probably due to the older age at the time of the interview.

The average cycle length was not related to PCB or DDE level within the four populations (Table II). However, when restrictions were made to minor deviations in cycle length (20–40 days), a positive association of DDE and cycle length was found in Poland, but not in any of the other countries.

Table I. Characteristics of the study population described with mean and 95% confidence interval, except for the grouped variables, which are presented as % of the group of interest or number of persons (*n*).

	Greenland, Inuits, $n = 454$	Sweden, Fishermen's wives, $n = 463$	Poland, Warsaw, $n = 203$	Ukraine, Kharkiv, $n = 374$
Outcomes				
Cyclus length (days)	30.0 (29.5; 30.6)	28.5 (28.0; 29.1)	30.1 (29.6; 30.7)	28.1 (27.7; 28.4)
Irregular cycles (%)	14	–	9	4
Short cycles (< = 24 days %)	2	5	1	10
Long cycles (> = 32 days %)	3	6	19	7
Exposure				
PCB-153 ng/g lipid	177 (158; 195)	174 (162; 186)	12 (11; 13)	33 (29; 36)
PCB-153 <50 ng/g lipid (*n*)	89	34	202	323
PCB-153 50–150 ng/g lipid (*n*)	184	202	1	49
PCB-153 >150 ng/g lipid (*n*)	181	220	0	2
DDE ng/g lipid	444 (406; 482)	2147(1788; 2506)	430(393; 467)	800 (745; 854)
DDE <370 ng/g lipid (*n*)	250	127	96	49
DDE 370–750 ng/g lipid (*n*)	127	91	84	165
DDE >750 ng/g lipid (*n*)	77	238	23	160
Covariates				
Age at menarche (years)	12.8 (12.7; 12.9)	12.9 (12.8; 13.0)	13.3 (13.2; 13.5)	13.0 (12.8; 13.1)
Age at pregnancy (years)	26.9 (26.4; 27.4)	29.2 (28.8; 29.7)[a]	28.8 (28.4; 29.2)	25.4 (25.0; 25.9)
BMI (kg/m^2)	24.5 (24.1; 24.9)	25.3 (25.0; 25.6)[b]	21.7 (21.2; 22.2)	21.6 (21.3; 21.9)
Parity (number of live born children)	1.4 (1.2; 1.5)	2.6 (2.5; 2.7)	0.1 (0.0; 0.1)	0.2 (0.2; 0.3)
Drinks/week	2.1 (1.6; 2.6)	1.3 (1.0; 1.5)	1.9 (1.6; 2.3)	0.7 (0.6; 0.8)
Smoking, %	79	30	17	21

[a]Age at last planned pregnancy; [b]Current body mass index. PCB-153: 2,2′,4,4′,5,5′-hexachlorobiphenyl which was used as a biomarker for PCB exposure. DDE: 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene, a metabolite which reflects long-term exposure to DDT.

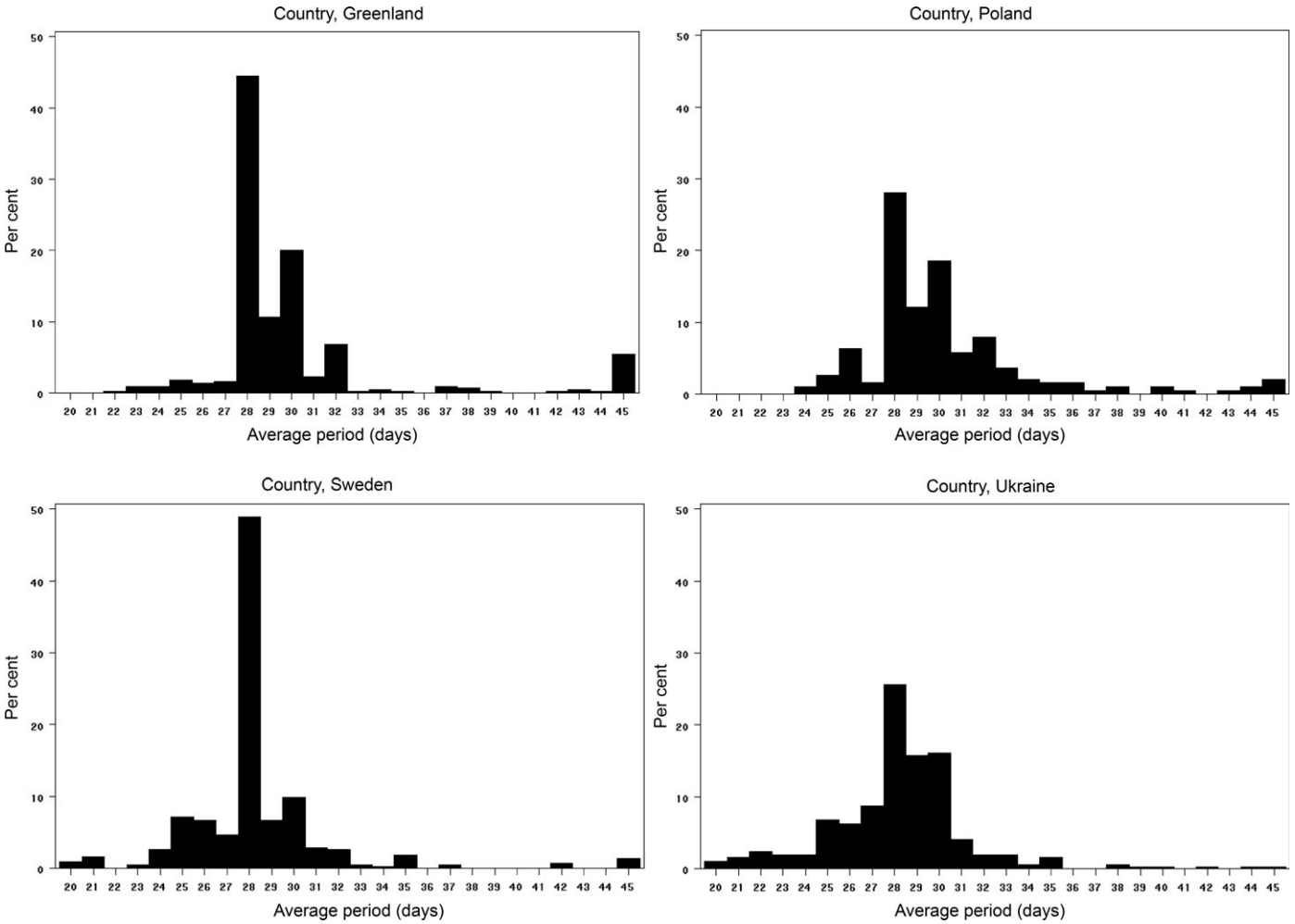

Figure 1: Distributions of reported average menstrual cycle length in the four countries
The lowest category includes all reporting ≤20 days cycle length and the highest includes all reporting ≥45 days of cycle length.

Table II. The association between chemical exposure and menstrual cycle characteristics (change in average cycle length in days for 10% increase in exposure and 95% CI).

Exposure	Greenland, Inuits ($n = 454$)	Sweden, Fishermen's wives ($n = 463$)	Poland, Warsaw ($n = 203$)	Ukraine, Kharkiv ($n = 374$)
PCB-153	−0.04 (−0.1; 0.03)	0.02 (−0.07; 0.12)	−0.05 (−0.1; 0.04)	0.01 (−0.04; 0.07)
DDE	−0.05 (−0.1; 0.03)	−0.03 (−0.02; 0.08)	0.01 (−0.09; 0.11)	0.01 (−0.06; 0.08)
PCB-153[a]	−0.01 (−0.03; 0.01)	−0.02 (−0.07; 0.04)	0.004 (−0.02; 0.10)	−0.02 (0.06; 0.03)
DDE[a]	−0.02 (−0.04; 0.01)	−0.01 (−0.03; 0.01)	**0.10 (0.03; 0.2)**	−0.03 (−0.08; 0.03)

Significant associations between cycles length and exposure are marked in bold. Controlled for female age at pregnancy, body mass index, parity, smoking and drinking. [a]Restricted to minor deviations in irregularity ($n = 1439$).

The associations of cycle length and exposure were not homogenous across populations, and therefore we were not able to make a combined estimate of the effects across populations.

The ORs for irregular cycles were not increased with increasing exposure to PCB or DDE in any of the three populations (outcome not investigated for the Swedish women; Table III). However, the risk for short cycles increased markedly in the Swedish population with increasing PCB and tended to do so with increasing DDE exposure (Table III).

A decreased risk for long cycles was observed in Greenland with increasing PCB and DDE exposure, but not in any of the other populations, whereas increased risks of long cycles was observed in Poland at higher DDE levels.

Discussion

In the present study, we found no consistent effects of PCB or DDE exposure on menstrual cycle length or irregularity across populations. However, within populations, some indications of altered cycle length was observed, including an increased risk of short cycles among Swedish fishermen's wives exposed to high levels of PCB, and for long cycles among Polish women

Table III. Logistic regression analysis of ORs for irregular, short and long cycles.

	Greenland, Inuits ($n = 454$)	Sweden, Fishermen's wives ($n = 463$)	Poland, Warsaw ($n = 203$)	Ukraine, Kharkiv ($n = 374$)
Irregular cycles				
PCB: <50 ng/g lipid	1.0 (ref)	–	1.0 (ref)	1.0 (ref)
50–150 ng/g lipid	0.7 (0.4; 1.3)	–	–	1.0 (0.2; 5.0)
>150 ng/g lipid	0.7 (0.4; 1.3)	–	–	–
Continuous	0.8 (0.7; 1.1)		1.1 (0.5; 2.3)	1.0 (0.4; 2.2)
DDE:<370 ng/g lipid	1.0 (ref)	–	1.0 (ref)	1.0 (ref)
370–750 ng/g lipid	0.6 (0.3; 1.2)	–	0.4 (0.2; 1.3)	0.6 (0.2; 2.6)
>750 ng/g lipid	0.5 (0.3; 1.0)	–	0.5 (0.1; 2.4)	0.4 (0.1; 2.0)
Continuous	0.9 (0.7; 1.1)	–	0.6 (0.3; 1.3)	1.2 (0.5; 3.2)
Short cycles (≤24 days)				
PCB: <50 ng/g lipid	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
50–150 ng/g lipid	2.1 (0.2; 18.2)	0.4 (0.04; 4.4)	–	1.6 (0.6; 4.1)
>150 ng/g lipid	1.2 (0.1; 12.7)	3.2 (0.4; 25.9)	–	–
Continuous	1.0 (0.5; 2.0)	**2.5 (1.2; 5.1)**	–	1.0 (0.6; 1.7)
DDE: <370 ng/g lipid	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
370–750 ng/g lipid	0.8 (0.1; 4.3)	1.0 (0.2; 4.7)	–	0.8 (0.3; 2.3)
>750 ng/g lipid	1.2 (0.2; 6.7)	2.4 (0.8; 7.8)	–	0.6 (0.2; 1.8)
Continuous	1.2 (0.6; 2.3)	1.3 (0.9; 1.8)	–	0.8 (0.4; 1.6)
Long cycles (≥32 days)				
PCB: <50 ng/g lipid	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
50–150 ng/g lipid	0.5 (0.2; 1.1)	0.5 (0.1; 1.8)	–	0.9 (0.2; 3.1)
>150 ng/g lipid	**0.4 (0.2; 0.9)**	0.7 (0.2; 2.6)	–	–
Continuous	**0.7 (0.5; 0.96)**	0.9 (0.5; 1.5)	1.0 (0.6; 1.6)	1.2 (0.6; 2.4)
DDE:<370 ng/g lipid	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
370–750 ng/g lipid	0.5 (0.2; 1.1)	0.7 (0.2; 2.4)	1.3 (0.6; 2.9)	0.9 (0.3; 2.8)
>750 ng/g lipid	0.5 (0.2; 1.5)	1.0 (0.4; 2.4)	**3.1 (1.1; 8.6)**	0.6 (0.2; 2.2)
Continuous	**0.7 (0.5; 0.99)**	0.9 (0.7; 1.3)	1.8 (0.95; 3.2)	0.8 (0.3; 1.7)

Irregular cycles is defined as ≥7 days of variation. Short cycles and long cycles are compared with normal cycles as a reference (24–32 days). Adjusted for female age at pregnancy, body mass index, parity, smoking and drinking. Significant deviations from unity in the logistic regressions are marked in bold.

exposed to high levels of DDE. However, in Greenland it seemed that high levels of PCB or DDE was protecting against long cycles.

The differences in menstrual cycle characteristics between countries should be interpreted with caution and may reflect differences in recruitment between populations. Especially the Swedish population differed by being asked about their menstrual characteristics several years after their last planned pregnancy and at an older age than the other populations, whereas the other populations were asked about the period they tried to become pregnant, when they were pregnant.

Possible explanations for the differences in the association between PCB and DDE exposure and menstrual cycle pattern between countries include differences in exposure profiles and exposure sources. The four populations represented a more than 10-fold difference in mean PCB concentration and a 5-fold difference in DDE concentration, with Greenland representing relative high exposure to both compounds, whereas the estimated concentration in the last planned pregnancy in Sweden was showing a high PCB exposure and very high DDE exposure, in Warsaw, Poland relatively high DDE exposure and very low PCB exposure was observed, and in Kharkiv, Ukraine relative high DDE and low PCB was found. Furthermore, PCB and DDE were strongly correlated in Greenland (0.92) and Sweden (0.87), but less so in Ukraine (0.51) and Poland (0.52) (Spearman correlation coefficients). Different exposure profiles and possibly opposite effects of PCB and DDE exposure on cycle length, may thus explain some of the observed findings. However, the exposure pattern does not entirely fit such a simple model. Therefore, exposure to PCB and DDE cannot alone explain the observed effects, but it should be noted that these organochlorines are highly correlated to a large number of other organochlorines, the distribution of which is not necessarily equal between countries. In a related study on some of the male partners of the participating women in the present study we found differences in endocrine disrupting effects of serum samples with high estrogenicity among the Polish men compared with the other populations, whereas a large proportion of the Greenlandic population had compounds inducing antiestrogenic effects in their blood (Bonefeld-Jorgensen *et al*., 2006). Thus, the complex interplay of a number of compounds present in the body may explain some of the observed effects but it should also be noted that differences in lifestyle, dietary habits and genetics between populations may induce different susceptibility to environmental induced alterations of menstrual cycles. In another study on the male partners of the women in the present study, we found similar protecting effects on a marker of male reproductive function (sperm DNA integrity) among Greenlanders, but not among the other European populations (Spano *et al*., 2005). The potential beneficial effects of omega 3 fatty acids originating from fatty fish on menstrual cycle stability is plausible due to the known endocrine effects of these essential compounds (Bhathena, 2000). Especially in the Greenlandic and the Swedish fishermen population, organochlorines are related to dietary seafood consumption (Jönsson *et al*., 2005) which may explain the apparent protection against long cycles in Greenland and shorter cycles in Sweden after organochlorine exposure and the opposite effect in the Polish population where organochlorines are not associated to seafood consumption to the same extent. Also in previous studies, shorter cycles have predominantly been

observed in studies where organochlorine exposure was mainly caused by fish consumption, (Mendola *et al*., 1997; Axmon *et al*., 2004; Windham *et al*., 2005), whereas longer cycles or no effects were found in populations not expected to consume large amount of fish (Chen *et al*., 2005; Cooper *et al*., 2005; Chao *et al*., 2007). However, in a study of Chinese textile workers with no information on fish consumption, shorter cycles were observed among the women exposed to high levels of DDT (Ouyang *et al*., 2005).

The largest previous epidemiological study of PCB and DDE exposure and menstrual cycle length and irregularity was performed among 2314 pregnant women included in the Collaborative Perinatal Project, a cohort study in the 1960's in 12 centers in the USA (Cooper *et al*., 2005). In that study, increasing PCB concentrations was related to increasing cycle length and both increasing PCB and DDE seemed to be related to an increased risk of having irregular menstrual cycles. Another large study of the effects of DDT and DDE exposure on menstrual cycles was recently performed among 466 Chinese textile workers (Ouyang *et al*., 2005), indicating increased risk of short cycles at high DDT and DDE exposure. All our exposure categories of DDE were included in the lowest exposure category in both of these studies, but the PCB exposure was in the same range as in the American study, although Cooper *et al*. measured the total of 11 PCB congeners which is ~3.6 times higher than the PCB-153 level we used (Toft *et al*., 2004). In the American study, menstrual cycle length was only significantly associated to PCB controlled for triglycerides and cholesterol, and not in the analysis presented as PCB in ng/g lipid, as we used in the present study. When we analyzed our results using the method with control for triglycerides and cholesterol, we still did not find any significant associations. As mentioned, the measurement of PCB in our study is restricted to PCB-153, whereas Cooper *et al*. (2005) measured 11 PCB congeners. In this study, PCB-153 was used as an index biomarker of total PCB exposure. There are several previous studies supporting this approach since high correlations between PCB-153 and several other PCB congeners have been observed (Grimvall *et al*., 1997; Glynn *et al*., 2000; Muckle *et al*., 2001). However, there are large differences in toxic and endocrine disrupting properties of the different PCB congeners (ATSDR, 2000) and due to different half-life of the different congeners the composition of congeners in a PCB mixture differs over time. Therefore the mixture the American population was exposed to in the 1960's may have been more toxic to the female reproductive function than the present exposure in populations where only the compounds with a long half-life are found in high concentrations.

The largest exposures to PCBs in human populations have been found in populations accidentally exposed to these compounds as e.g. the Yucheng cohort in Taiwan exposed to PCBs via contaminated cooking oil. In a study of 356 exposed women and 312 controls, an increased incidence of abnormal menstrual flow was reported among the exposed women, but there was not an increased proportion of women with irregular menstrual cycles among the exposed women (Yu *et al*., 2000). However, when evaluating the apparent lack of strong disturbing effects on menstrual cycles in this population, it should be noted that the interviews on menstrual cycles were performed 14–15 years after the accidental exposure episode, when the individual PCB exposure were significantly reduced, but still about seven times higher than the background exposure level in Taiwan (Guo *et al*., 1997).

Other studies have shown somewhat conflicting results on the effects of PCB and DDE on menstrual cycle length and regularity (Mendola *et al*., 1997; Axmon *et al*., 2004; Chen *et al*., 2005; Windham *et al*., 2005; Chao *et al*., 2007). In some of these studies (Mendola *et al*., 1997; Axmon *et al*., 2004), PCB exposure was estimated from fish consumption or fishermen cohort affiliation, which only gives an imprecise estimate of the actual exposure. When Axmon *et al*. (2004) evaluated the effects of measured PCB-153 in a subset of their population, they found no significant effects.

In the remaining studies, the power to detect any changes is limited since only 47–119 women were included in the studies (Chen *et al*., 2005; Windham *et al*., 2005; Chao *et al*., 2007). All studies performed so far on menstrual cycle, including the present study, have used retrospective self-report of menstrual cycles and therefore some misclassification of menstrual cycle length is likely (Small *et al*., 2007). In the present study, the risk of misclassification of the menstrual cycle data is especially high for the Swedish fishermen recalling menstrual periods several years back in time (median 19 years recall time), which may be unreliable (Bean *et al*., 1979). The misclassification of exposure is also high in the studies relying on fish consumption or cohort affiliation, but is not considered a problem in the present study, except for the Swedish fishermen's wives, where the exposure estimation is dependent on the assumptions in the model. The potential misclassification of the outcome and/or exposure in the present study will most likely be non-differential, causing an underestimation of any true effects.

The participation rates differed markedly between countries from a high of 90% in Greenland to a low of 26% in Kharkiv. The low participation rate in Kharkiv opens the possibility for selection of participants with altered fertility compared with the general population, but none of the participants had previous knowledge of their exposure to persistent organochlorines, limiting the risk of differential participation based on both exposure and outcome. Therefore, the overall analysis of the relation between organochlorine exposure and menstrual disturbances are still valid within the included populations, but the generalizability of the results may be limited, especially in the countries with low participation rate.

The strengths of the present study include measurements of both PCB and DDE and menstrual cycles with the same methods in four populations, with large exposure contrasts both within and between populations. If PCB and DDE have a substantial effect on menstrual cycles it is expected to be observed in all of the four populations, or at least in the populations with the highest level of exposure, unless PCB and DDE have opposite effects.

Since we did not observe consistent effects of PCB or DDE on menstrual cycles, and even opposite associations in some of the countries, it is unlikely that PCB and DDE are the main cause of these effects even in populations with relative high level of exposure. We included the known confounders in the analysis, but we are not able to determine if other confounding

factors may have influenced the results or if differences in genetics and uncontrolled life style factors had an influence on the observed effects.

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