Time to pregnancy as a function of male and female serum concentrations of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p′-DDE)


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BACKGROUND: Persistent organochlorine pollutants (POP) may affect both the female and male reproductive system in animals as well as in humans. METHODS: Blood samples were collected from pregnant women and their partners from Greenland, Warsaw and Kharkiv, and from a cohort of Swedish fisherman’s wives. Blood samples were analysed for 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p′-DDE). Information on the participants’ fertility, measured as time to pregnancy (TTP), was collected. In total, 778 men and 1505 women were included in the analyses. RESULTS: The data from Warsaw, Kharkiv and the Swedish fishermen’s wives indicated no effect of either male or female exposure to POP on TTP. However, among men and women from Greenland, there seemed to be an association between serum concentrations of CB-153 and p,p′-DDE and prolonged TTP. Due to the strong intra-individual correlation between CB-153 and p,p′-DDE in the Greenlandic population, it was not possible to determine whether the risk was associated with CB-153 or p,p′-DDE or was an interaction between the two compounds. CONCLUSIONS: The overall results of the present study create a somewhat ambiguous pattern, but give some support to the idea that dietary POP exposure might be harmful for couple fertility.

Key words: female exposure/fertility/male exposure/persistent organochlorine pollutants/seafood consumption

Introduction

Persistent organochlorine pollutants (POP), such as polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and pesticides such as dichlorodiphenyl trichloroethane (DDT), have been released into the environment, mainly since the Second World War. PCB and DDT were restricted or totally banned in most countries during the 1970s and 1980s, but DDT for example is still used in some areas for malaria vector control. The lipophilic POP are highly resistant to both abiotic and biotic degradation, and are transported long distances by weather systems. POP have been detected in human blood, adipose tissue and breast milk worldwide (Wicklund-Glynn et al., 1996; Gladen et al., 1999b).

Several PCB congeners and DDT/DDT have weak agonistic or antagonistic effects on several hormonal systems (Kelce et al., 1998; Bonefeld-Jorgensen et al., 2001; Portigal et al., 2002; Schrader and Cooke, 2003; Scippo et al., 2004). Studies on wildlife and laboratory animals show that exposure to PCB and p,p′-DDE can adversely affect reproductive and endocrine functions (Rosselli et al., 2000; Faroon et al., 2001; Gray et al., 2001; Guillette and Gunderson, 2001; Norgil Damgaard et al., 2002; Skaare et al., 2002; Teilmann et al., 2002; Toppari, 2002; Safe, 2004; Sharpe and Irvine, 2004; Toft et al., 2004).

The term fecundability denotes the monthly (or per cycle) chance of conception in the absence of contraception. The fecundability can be estimated by assessing the time (or number of menstrual cycles) that elapses between discontinuance of contraception and clinical recognition of a pregnancy, i.e. the time to pregnancy (TTP) (Weinberg and Gladen, 1986). Thus, TTP is a direct and functional measure of a couple’s fertility that can be assessed with high reliability and accuracy in epidemiological fertility studies in populations where pregnancy planning is prevalent (Baird et al., 1986; Joffe, 1989). It should be noted that TTP does not indicate if a delayed conception is caused by male or female factors and that early embryonal
loss that escapes clinical detection cannot be distinguished from
missing fertilization.

Several studies have been carried out to assess a possible
association between exposure to POP on one hand and TTP
and conception delay (i.e. TTP longer than a pre-determined
limit) on the other. The majority of these studies have been
done in populations for which the major exposure source was
through consumption of contaminated fish. Among these,
some studies have reported a possible association between
exposure and outcome (Buck et al., 2000; McGuinness et al.,
2001), whereas others have failed to replicate these results
(Buck et al., 1997, 1999; Axmon et al., 2000, 2001, 2002,
2004b). With respect to DDT exposure, spouses of male DDT
applicators have been found to have impaired fecundity (Cocco
et al., 2005).

The present study is a part of a collaborative research project
funded by the EU (Inuendo; www.inuendo.dk) which aims to
detect and characterize the impact of dietary POP on
human fertility in an epidemiological setting spanning great
contrasts in POP exposure. Cohorts have been established in
three European countries—Sweden, Poland and Ukraine—
together with cohorts of Inuits from Greenland. We have chosen
to use 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) as a biomar-
ker for POP exposure, because it correlates very well (r ≥ 0.98)
with both total PCB concentration in plasma and serum from
Swedish subjects (Grimvall et al., 2004b). With respect to DDT exposure, spouses of male DDT
applicators have been found to have impaired fecundity (Cocco
et al., 2005).

Materials and methods

Subjects and sampling

Between June 2002 and May 2004, we recruited pregnant women and
their male spouses in Greenland, Warsaw and Kharkiv respectively
and Swedish fishermen’s wives for interviews and blood sampling.
The number of men and women differed in the three cohorts where
both genders were included. This was due to reluctance to participate,
or to the father being unknown.

A detailed description of recruitment process and participation has
been given elsewhere (Jönsson et al., 2005; Toft et al., 2005). Briefly,
the same questionnaire was used for participants from Greenland,
Warsaw and Kharkiv (prospective pregnancy-based studies enrolling
couples consecutively during antenatal care visits), and a slightly
modified version was used for the Swedish fishermen’s wives
(a retrospective population-based study). A general criterion for eligi-
bility was that the participants were born in the country of study
and ≥18 years of age. Altogether 2269 couples/fishermen’s wives
were interviewed. The Inuits and Kharkiv women were earlier in
their pregnancies at the time of the interview (average days pregnant
171 and 166 respectively) than the women from Warsaw (average
232 days pregnant). Depending on different procedures for recruit-
ment, the participation rate differed between the four countries, with
low rates in Kharkiv (26%) and Sweden (39%), but higher in Warsaw
(68%) and among the Inuits (90%).

The study was approved by the local ethics committees represent-
ing all participating populations and all subjects signed an informed
consent.

Outcome

The questions used to establish the TTP of each couple have been
described previously (Toft et al., 2005). Of 2269 interviewed women,
1722 (376 from Warsaw, 307 from Kharkiv, 520 Inuits, and 519
Swedish fishermen’s wives) provided a valid TTP. The major reason
for not providing a TTP among the pregnant women was that the preg-
nancy was unplanned (9% among the Inuits, 19% among the Warsaw
women, and 49% among the Kharkiv women). For the Swedish fish-
ermen’s wives, the major reason was failure to remember.

Exposure

Collection of blood samples

Blood samples were drawn from a cubital vein into 10 ml vacuum tubes
for serum collection without additives (Becton Dickinson, Moylan,
France). After cooling to room temperature the tubes were centrifuged at
4000 g for 15 min. Serum was transferred with ethanol-rinsed Pasteur
pipettes to ethanol-rinsed brown glass bottles (Termometerfabriken,
Gothenburg, Sweden). A piece of aluminium foil was placed on top of the
bottles which were then sealed. Sera were stored at −20°C until shipment,
but it was allowed to keep samples in a refrigerator for up to 4 days.

Determination of CB-153 and p,p′-DDE in serum

All analyses of CB-153 and p,p′-DDE in serum were performed at the
Department of Occupational and Environmental Medicine in Lund,
Sweden, applying solid phase extraction using on-column degradation
of the lipids and analysis by gas chromatography mass spectrometry
as previously described (Richthoff et al., 2003; Rignell-Hydbom
et al., 2004; Jönsson et al., 2005). Levels of detection, coefficients of
variation and participation in quality control programmes have been
described in detail elsewhere (Jönsson et al., 2005).

Determination of lipids by enzymatic methods

Serum concentrations of triglycerides and cholesterol were deter-
mined by enzymatic methods as described elsewhere (Jönsson et al.,
2005). The total lipid concentration in serum (g/l) was calculated by
the following equations (Rylander et al., 2005):

Men: Total = 0.96 + 1.28*(triglycerides + cholesterol)
Women: Total = 1.13 + 1.31*(triglycerides + cholesterol).

Backwards estimation of the exposure biomarkers for the Swedish
fishermen’s wives

To enable a comparison between the current serum concentrations of
CB-153 and p.p′-DDE for the other populations, estimated past concen-
trations of CB-153 and p,p′-DDE, modelled from the current lev-
els, were used for the Swedish women (Rylander et al., 1998; Axmon
et al., 2001). Previous experience has suggested that for both CB-153
and p,p′-DDE, breastfeeding for <6 months reduces the body’s burden
by 20%, whereas breastfeeding for >6 months reduces the body's burden by 30% (Axmon et al., 2001; Axmon and Rignell-Hydbom, 2006). Furthermore, for CB-153 we assumed that the human biological half-life was 5 years during non-lactational periods, and that there has been a 3% yearly reduction of CB-153 in fatty fish from the Baltic Sea from 1976 and onwards (Axmon et al., 2001). For p,p′-DDE it has been found reasonable to assume that the human biological half-life is 8 years, and that there has been a 20% yearly decrease of the compound in Baltic Sea fatty fish between 1971 and 1981, and a 9% yearly decrease thereafter (Axmon and Rignell-Hydbom, 2006).

**Measures of exposure**

Among the couples for which a valid TTP was available, exposure biomarkers were determined in serum from 1505 women (207 from Warsaw, 293 from Kharkiv, 497 Inuits, and 508 Swedish fishermen's wives) and 716 men (203 from Warsaw, 129 from Kharkiv, and 384 Inuits). In total, four measures of exposures were used in the present study: female serum concentrations of CB-153 and p,p′-DDE, and male serum concentrations of CB-153 and p,p′-DDE. In countries where both male and female biomarkers were available (all but Sweden), there were only weak associations between male and female CB-153 and p,p′-DDE respectively (both rs < 0.3). Among the Inuit women and the Swedish fishermen’s wives, the correlations between female CB-153 and p,p′-DDE were markedly higher (rs = 0.92 and 0.87 respectively) than among the women from Warsaw and Kharkiv (rs = 0.49 and 0.54 respectively). Similarly the correlation between CB-153 and p,p′-DDE was higher among the Inuit men (rs = 0.93) than in Warsaw men (rs = 0.20) and Kharkiv men (rs = 0.42).

**Non-respondents, excluded or missing data**

There were no differences with respect to age and parity between female Inuit respondents and non-respondents, neither did the Swedish respondents differ from the non-respondents with respect to age (since the Swedish part of the study was retrospective, parity was not an issue). In Kharkiv, the average age was slightly lower among non-participating women (22.8 versus 24.9 years), although they comprised fewer primiparae (26 versus 20%). There were no data available on the non-participants from Warsaw. A more detailed description of the non-respondent analysis has been presented elsewhere (Toft et al., 2005).

The Inuits with unplanned pregnancies had slightly lower male serum concentrations of CB-153 (median 140 versus 210 ng/g lipid) and p,p′-DDE (median 417 versus 600 ng/g lipid) compared to the couples included in the TTP analyses. For female Inuits and for men and women from Kharkiv and Warsaw, the relative difference in median serum concentrations ranged between 0 and 20%.

**Possible confounders**

In the interview, the women were asked about several potential confounders, including age at conception, parity, prior number of induced abortions, frequency of intercourse (daily, at least once a week, two to four times a month, or less than twice a month) during the time she and her partner attempted pregnancy, smoking habits (yes/no) during the same time period, as well as method of birth control used prior to the time when she tried to conceive. The women from Greenland, Warsaw and Kharkiv were asked about their pre-pregnancy height and weight, whereas the Swedish women were asked about their current height and weight. Using this information, a body mass index (BMI) was calculated. Women were considered to have had a urogenital inflammation if they said they had ever been diagnosed with one of the following diseases: pelvic inflammatory disease, gonorrhoea or chlamydial infection, other sexually transmitted diseases, or pelvic infections after a previous pregnancy. Furthermore, urogenital surgery was defined as operations due to ovarian cysts, fibroids, myomas, endometriosis, or other operations of the Fallopian tubes or ovaries, or chemical or radiation therapy because of urogenital cancer. Women with either thyroid disease or diabetes mellitus were considered as having a chronic disease.

The men were asked about their age and smoking habits (yes/no). Furthermore, all men were asked if they had ever had epididymitis, gonorrhoea or chlamydial infection, or if they had had mumps as an adult. These diseases were grouped as urogenital infection. Men who stated that they had undergone surgery for varicose veins of the scrotum, testicular torsion, or testicular cancer, or who had ever been treated for cryptorchidism were considered as having had urogenital surgery.

When the measure of exposure was either female CB-153 or female p,p′-DDE, we considered age at conception, frequency of intercourse, BMI (continuous), smoking (yes/no), parity, previous induced abortions (two or more), use of oral contraceptives prior to when trying to conceive, urogenital surgery, urogenital inflammation, and chronic diseases as potential confounders. When the measure of exposure was male CB-153 or male p,p′-DDE, both the man’s and woman’s age and smoking habits, as well as frequency of intercourse, male urogenital disorders, and male urogenital surgery were considered potential confounders.

**Statistics**

**Measures of exposure**

Since there were no previous suggestions of a linear relationship between the biomarkers and TTP, we chose to categorize the exposure variables (Axmon et al., 2001, 2004b). Using the full exposure range, the female serum concentrations of CB-153 and p,p′-DDE were trichotomized in such a way that the lowest category (i.e. the reference category) for each country contained 240 observations (Tables I and III). The rest of the participants were then divided into two approximately equal parts. However, when using this strategy, all Warsaw women but one were classified as having low exposure to CB-153, and could therefore not be included in analyses for the full exposure range. Thus, we performed a further trichotomization within the low exposure range (i.e. all women who were classified as ‘low’ in the original grouping). This second trichotomization into three equally sized groups within the low exposure range—‘low–high’, ‘low–medium’ and ‘low–low’—was applied for the cohorts from Warsaw and Kharkiv only (Table II).

Similarly to the measures of female exposure, CB-153 and p,p′-DDE serum levels in men from Greenland, Warsaw and Kharkiv were trichotomized in such a way that the lowest category contained ≥40 observations (Tables I and III). Again, there were only a few Warsaw and Kharkiv men in the medium and high exposure groups, and as for the women, a second trichotomization was performed within the low exposure range of CB-153 (‘low–high’, ‘low–medium’ and ‘low–low’; Table II).

To investigate a possible interaction between male and female exposure, two variables were created using the gender-specific medians of the two biomarkers (excluding the Swedish fishermen’s wives): low male and female exposure (reference category), low female and high male exposure, high female and low male exposure, and high male and female exposure.

**Analysis of TTP data**

Logistic regression was employed to estimate the association between exposure and TTP. For each comparison, a fecundability ratio (FR) was calculated from the odds ratio (OR) for pregnancy of each time interval (1–1.9, 2–2.9 months, etc.). In the analyses, TTP was censored at 12 months. All analyses were stratified on the country in which the data was collected.
Handling potential confounders

Multivariate analyses were performed including the potential confounders described above. When selecting confounders for the multivariate analyses, the change in estimate method suggested by Greenland (1989) was used. Starting with the univariate model, variables were entered into bivariate and multivariate models if they changed the effect estimate by ≥10%.

For some of the potential confounders, e.g. smoking and frequency of intercourse, there was a large number of missing data. In order to ensure that the inclusion of these variables was a way to deal with

Table I. Fecundability ratios (FR) with 95% confidence intervals (CI) in relation to the full exposure range for male and female serum concentrations of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153)

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Greenland</th>
<th>Swedish fisherman’s wivesa</th>
<th>Warsaw</th>
<th>Kharkiv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female exposure</td>
<td>Male exposure</td>
<td>Female exposure</td>
<td>Male exposure</td>
</tr>
<tr>
<td></td>
<td>FR (95% CI)</td>
<td>FR (95% CI)</td>
<td>FR (95% CI)</td>
<td>FR (95% CI)</td>
</tr>
<tr>
<td>Low (reference)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Medium</td>
<td>0.85 (0.64–1.14)</td>
<td>0.78 (0.51–1.19)</td>
<td>0.86 (0.65–1.15)b</td>
<td>0.82 (0.60–1.10)b</td>
</tr>
<tr>
<td>High</td>
<td>0.73 (0.54–0.97)</td>
<td>0.77 (0.51–1.16)</td>
<td>0.68 (0.54–1.05)b</td>
<td>0.84 (0.60–1.15)</td>
</tr>
</tbody>
</table>

For female data: low <56 ng/g lipid, medium 56–150 ng/g lipid and high >150 ng/g lipid; for male data: low <70 ng/g lipid, medium 70–230 ng/g lipid and high >230 ng/g lipid.

The serum concentrations of CB-153 for the Swedish fisherman’s wives were estimated from date of sampling to the time when the woman tried to conceive in her latest planned pregnancy (median time difference 23 years; range 1–44 years).

Adjusted for maternal age (one, two and two subjects missing in the low, medium and high exposure categories respectively).

Table II. Fecundability ratios (FR) with 95% confidence intervals (CI) in relation to the low exposure range for male and female serum concentrations of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153)

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Warsaw</th>
<th>Kharkiv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female exposure</td>
<td>Male exposure</td>
</tr>
<tr>
<td></td>
<td>FR (95% CI)</td>
<td>FR (95% CI)</td>
</tr>
<tr>
<td>Low–low</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Low–medium</td>
<td>1.11 (0.74–1.66)</td>
<td>1.11 (0.77–1.60)</td>
</tr>
<tr>
<td>Low–high</td>
<td>na2</td>
<td>0.60 (0.31–1.16)</td>
</tr>
</tbody>
</table>

For female exposure: low <15 ng/g lipid, medium 15–30 ng/g lipid and high 30–56 ng/g lipid; for male exposure: low <18 ng/g lipid, medium 18–32 ng/g lipid and high 32–70 ng/g lipid.

Adjusted for frequency of intercourse (five, three and two subjects missing in the low–low, low–medium and low–high exposure categories respectively).

Adjusted for frequency of intercourse, parity, and maternal age at conception (20, 42 and 44 subjects missing in the low–low, low–medium and low–high exposure categories respectively).

Adjusted for maternal and paternal age, and frequency of intercourse (37, seven and one subjects missing in the low–low, low–medium, and low–high exposure categories respectively).

Not analysed because of too few observations.

Table III. Fecundability ratios (FR) with 95% confidence intervals (CI) in relation to female serum concentrations of 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p′-DDE)

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Greenland</th>
<th>Swedish fisherman’s wivesa</th>
<th>Warsaw</th>
<th>Kharkiv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(%)</td>
<td>FR (95% CI)</td>
<td>n(%)</td>
<td>FR (95% CI)</td>
</tr>
<tr>
<td>Low (&lt;370 ng/g lipid)</td>
<td>284</td>
<td>1.00</td>
<td>135 (27)</td>
<td>1.00</td>
</tr>
<tr>
<td>Medium (370–750 ng/g lipid)</td>
<td>136</td>
<td>0.90 (0.70–1.16)</td>
<td>100 (20)</td>
<td>1.22 (0.87–1.71)</td>
</tr>
<tr>
<td>High (&gt;750 ng/g lipid)</td>
<td>77</td>
<td>0.68 (0.49–0.94)</td>
<td>273 (54)</td>
<td>1.02 (0.78–1.34)</td>
</tr>
</tbody>
</table>

The serum concentrations of CB-153 for the Swedish population were estimated from date of sampling to the time when the woman tried to conceive in her latest planned pregnancy (median time difference 23 years; range 1–44 years).

Adjusted for maternal age at conception (2, 3 and 0 subjects missing in the low, medium and high exposure categories respectively).

Adjusted for frequency of intercourse (three, six and one subjects missing in the low, medium and high exposure categories respectively).

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confounding effects rather than making a selection of data, the change-in-estimate was assessed with respect to those crude FR that were obtained when excluding observations with missing information on the potential confounder investigated.

We allowed for different sets of confounders for the country-specific analyses.

Handling multiple measures of exposure
We evaluated the impact on TTP of four measures of exposure, which could be considered as acting as confounders for each other. Therefore a stepwise technique was used to determine the independent impact of each measure. In the first step, each exposure measure was considered separately with no consideration taken for the other three measures. When a final model (i.e. a model including the appropriate confounding factors) had been determined for each measure of exposure, the other measures of exposures were added in a second step to the model one at a time and their effect on the FR was established. Only if the change in FR was >10% was the added measure of exposure considered to have an impact on the FR. However, if the correlation coefficient between two measures of exposure was >0.80, we did not perform the second step.

When considered as exposure measures, the serum concentrations of CB-153 and p,p′-DDE were always used in their trichotomized form. However, when considered as potential confounders, the serum concentrations were used in their continuous form. The reason for using two different forms was the high correlations between CB-153 and p,p′-DDE for some of the countries.

Results
Female serum concentrations of CB-153 and TTP
Among those who had provided a TTP, the Swedish fishermen’s wives had the highest serum concentrations of CB-153 [median 150 ng/g lipid, 95% range (2.5 to 97.5 percentile) 34–530], followed by the Inuits (median 110 ng/g lipid, 95% range 11–690). The women from Kharkiv had the highest median concentrations of CB-153 (median 210 ng/g lipid, 95% range 2.4–29) and Warsaw (median 11 ng/g lipid, 95% range 2.4–29) had substantially lower serum concentrations of CB-153.

The results for the Inuit women suggested that within the full exposure range, higher serum concentration of CB-153 was associated with longer TTP (i.e. decreased FR), but this was not supported by the results from the Swedish fishermen’s wives (Table I). Within the low exposure range, the data from the women from Kharkiv gave a weak and non-statistically significant support for an association between CB-153 and longer TTP, but this was not supported by the Warsaw data (Table II). Potential confounders described in Materials and methods, but not included in Tables I and II, were evaluated using the change in estimate method, but did not change the FR any further.

In the analyses for the full exposure range, the models were not adjusted for female p,p′-DDE since the correlations with female CB-153 were too high. Also, the FR for the Swedish fishermen’s wives were not adjusted for male biomarker concentrations, since no men were included from Sweden. Adjusting the Inuit FR for male CB-153 and p,p′-DDE did not change the FR in the final full exposure range models.

In the low exposure range, the risk associated with the Kharkiv low–high exposure category was even more pronounced when including female p,p′-DDE exposure (FR 0.69; 0.38–1.26). The subgroup of couples from Kharkiv for which both the man and woman had provided blood samples had decreased FR compared to the whole group (data not shown). The FR were further decreased when including male exposure to CB-153 (FR 0.28, 0.11–0.71 for the low–medium and FR 0.26, 0.09–0.71 for the low–high exposure group). In the data from Warsaw, only four couples were missing male exposure. Excluding these couples did not affect the FR, neither did adding the female p,p′-DDE or male CB-153 or p,p′-DDE (data not shown).

There were no indications of an interaction between female and male serum concentrations of CB-153 (data not shown).

Female serum concentrations of p,p′-DDE and TTP
As with CB-153, the Swedish fishermen’s wives with valid TTP had the highest concentrations of p,p′-DDE (median 820 ng/g lipid, 95% range 92–14000). The women from Kharkiv had slightly lower concentrations (620 ng/g lipid, 95% range 230–1800), whereas the lowest median concentrations were found for the women from Warsaw (median 360 ng/g lipid, 95% range 100–1100) and Greenland (median 300 ng/g lipid, 95% range 26–1700).

There was a prolonged TTP for the highly exposed Inuits (Table III). The effect remained after adjusting for the relevant confounders. In the three other populations, no effect was found of serum concentrations of p,p′-DDE on TTP. Potential confounders that are not included in Table III were evaluated using the change in estimate method, but did not change the FR any further.

The Swedish and Inuit FR were not adjusted for female CB-153 levels due to too high inter-correlations (see above). Adjustment for male biomarkers did not change the FR for Greenland or Warsaw notably, whereas including male concentrations of p,p′-DDE raised the FR among the Kharkiv women (FR 1.16, 0.39–3.44 for the medium and FR 1.45, 0.48–4.41 for the high exposure group).

There were no indications of an interaction between male and female serum concentrations of p,p′-DDE (data not shown).

Male serum concentrations of CB-153 and TTP
Among the couples with valid TTP, the Inuit men had substantially higher serum concentrations of CB-153 (median 210 ng/g lipid, 95% range 34–1300) than both Kharkiv (median 44 ng/g lipid, 95% range 14–160) and Warsaw (median 18 ng/g lipid, 95% range 4.3–46) men.

The full exposure range analyses of the Inuit data and the low exposure range analyses of the Kharkiv data gave weak indications of a prolonged TTP associated with male CB-153 exposure (Tables I and II). However, these results were not supported by the low exposure range analyses of the Warsaw data, or by the adjusted FR presented in Tables I and II. Potential confounders described in Materials and methods, but not included in Tables I and II, were evaluated using the change in estimate method, but did not change the FR any further.

The Inuit FR were not adjusted for male p,p′-DDE since the correlation between the two biomarkers was too high. However, the FR for the high Inuit exposure category shifted towards unity when the final model was adjusted for female
Fecundability ratios (FR) with 95% confidence intervals (CI) in relation to male serum concentrations of 1,1-dichloro-2,2-bis(p,p’-DDE) (FR 0.89, 0.58–1.37) or female CB-153 (FR 0.88, 0.57–1.35), whereas in the low exposure range, inclusion of female CB-153 (FR 0.71, 0.21–2.41) or p,p’-DDE (FR 0.74, 0.22–2.54) resulted in lowered FR for the Kharkiv low–medium category, and inclusion of male p,p’-DDE lowered the FR for both the low–medium (FR 0.76, 0.23–2.55) and low–high (FR 0.59, 0.18–1.99) category.

Male serum concentrations of p,p’-DDE and TTP

The men from Kharkiv had the highest serum concentrations of p,p’-DDE (median 920 ng/g lipid, 95% range 360–2900), whereas the Inuits (median 600 ng/g lipid, 95% range 64–2700) and Warsaw men (median 520 ng/g lipid, 95% range 200–1300) had lower median concentrations.

After having adjusted for the relevant confounders, there were no obvious indications of a prolonged TTP associated with increasing serum concentrations of p,p’-DDE in any of the populations (Table IV). Potential confounders described in Materials and methods, but not included in Table IV, were evaluated using the change in estimate method, but did not change the FR any further.

The Greenlandic final model was not adjusted for male CB-153 due to a high correlation between the two biomarkers. However, including female p,p’-DDE (FR 1.19; 0.84–1.69) or female CB-153 (FR 1.15; 0.82–1.63) into the model raised the FR for Inuit men in the high exposure category. For the Polish men, inclusion of male CB-153 concentrations shifted the FR for the high exposure group towards unity (FR 0.81; 0.25–2.61). None of the other FR in Table IV were affected by the inclusion of the other measures of exposure.

Discussion

There seems to be a weak but consistent association between the POP biomarkers and prolonged TTP in the Inuit population. A similar pattern was seen for the Kharkiv cohort with respect to CB-153, despite considerably lower exposure levels compared to the Inuit and Swedish cohorts. In contrast, no prolonged TTP in relation to female p,p’-DDE was seen in Kharkiv, even though the exposure levels were similar to those among the Inuit women. The results from the Swedish fishermen’s wives and the Warsaw cohorts did not support any association between POP exposure and prolonged TTP. Since the results from the four countries differed, no joint analyses were performed.

The POP biomarker CB-153 correlates very well with, for example, total PCB concentration in serum from Swedish subjects and Inuits from Greenland (Grimvall et al., 1997; Glynn et al., 2000; Deutch et al., 2003). We have previously shown that the exposure sources for CB-153 and p,p’-DDE differed between the four participating countries (Jönnson et al., 2005). Whereas for the Inuits and Swedish fishermen’s wives, consumption of contaminated seafood was an important source, dietary or other exposure sources of relevance in Kharkiv and Warsaw have not been clarified. Whether CB-153 was the optimal choice as proxy biomarker for the overall POP exposure for the populations in Warsaw and Kharkiv is not known. This has to be considered when interpreting the results of the present study although differences in exposure profiles hardly explain the inconsistent findings with respect to CB-153 and TTP among Inuits and Swedish fishermen’s families.

In three of the cohorts, blood samples were collected during the pregnancy. However, for the Swedish fishermen’s wives, blood samples were collected several years (median 19 years) after pregnancy. To estimate serum concentrations for the Swedish fishermen’s wives at the time of the pregnancy, we used a complex decay model, which has been recommended when repeated measurements are not available (Karmaus et al., 2004). A certain degree of exposure misclassification compared with the ‘true’ serum levels during pregnancy is, however, unavoidable using backward estimations. This misclassification is considered to be non-differential, which will tend to drive the FR towards unity. It should also be noted that the estimated past POP levels for the Swedish fishermen’s wives enabled comparisons between all four countries with respect to absolute exposure levels during pregnancy.

Whether or not a couple trying to become pregnant conceives in a given menstrual cycle depends on several factors. Nevertheless, TTP is a measure that takes into account effects on both the male and female reproductive capacity, as well as the early survival of the fetus. It has been recognized as a useful tool for assessing reproductive effects of exposures in general (Joffe, 1989) and environmental exposure in particular (Baird et al., 1986).

The Swedish data differ from the other data by being collected retrospectively. The median recall time for the Swedish

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Table IV. Fecundability ratios (FR) with 95% confidence intervals (CI) in relation to male serum concentrations of 1,1-dichloro-2,2-bis(pchlophenyl)-ethylene (p,p’-DDE)

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Greenland</th>
<th>Warsaw</th>
<th>Kharkiv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>FR (95% CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Low (&lt;740 ng/g lipid)</td>
<td>225 (59)</td>
<td>1.00</td>
<td>153 (75)</td>
</tr>
<tr>
<td>Medium (740–1200 ng/g lipid)</td>
<td>69 (18)</td>
<td>0.87 (0.62–1.21)</td>
<td>42 (21)</td>
</tr>
<tr>
<td>High (&gt;1200 ng/g lipid)</td>
<td>90 (23)</td>
<td>0.87 (0.64–1.18)</td>
<td>8 (4)</td>
</tr>
</tbody>
</table>

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aAdjusted for paternal age at conception (three observations missing in the low exposure category).
bAdjusted for frequency of intercourse (five, three and one subjects missing in the low, medium and high exposure categories respectively).
cAdjusted for frequency of intercourse and maternal age (19, 13 and 11 subjects missing in the low, medium and high exposure categories respectively).
fishermen’s wives was 19 years (range 0–38 years). However, despite indications that women on a group level have a remarkably good recall of TTP (Joffe, 1989) and there were no indications of a more pronounced digit preference (i.e. a bias that favours certain TTP, such as 3, 6 and 12 months) among the Swedish fishermen’s wives compared to women from the other cohorts (data not shown), we cannot rule out a certain misclassification of TTP due to the retrospective study design for the Swedish fishermen’s wives. Nevertheless there is no reason to suspect that the POP exposure level has affected the response to the TTP questionnaire. Thus, misclassification of outcome is considered to be non-differential, which will tend to drive the FR towards unity.

Women whose pregnancies originally were unplanned may change their feelings about the pregnancy once it is recognized. These women tend to report their TTP as 1 month (Weinberg et al., 1994). To control for this ‘birth control bias’, it has therefore become standard to re-analyse the material, excluding first month conceptions. In the present material, 154 (31%) Greenlandic, 213 (42%) Swedish, 49 (24%) Warsaw, and 62 (21%) Kharkiv women stated that their TTP was 1 month (or less). Excluding them would result in a severe loss of data. Furthermore, there is no reason to believe that a woman’s inclination to report an unplanned pregnancy as planned should in any way be related to her or her partner’s serum concentrations of CB-153 or p,p’-DDE. Thus, we refrained from controlling for birth control bias in the present study.

The exposure biomarkers in the present study accumulate in the adipose tissue, and the most important way of reducing the body burden is to breastfeed. Thus, it would be of interest to analyse pregnancies of parity 1 only for the association between POP and TTP. However, restricting the analyses to parity 1 pregnancies would result in the loss of 438 (88%) Greenlandic, 482 (95%) Swedish, 48 (23%) Warsaw, and 115 (39%) Kharkiv women, i.e. again a severe reduction of data. Therefore, no such subgroup analyses were performed.

In Kharkiv, Warsaw and Greenland, pregnant women were enrolled in the study, whereas in the Swedish setting, women were asked about their latest planned pregnancy. In both cases, women who failed to conceive were excluded from the study. Thus, if the highest exposed women had completely failed to conceive, rather than experiencing a long TTP, the study design would cause us to underestimate the effect of exposure. However, we do not expect such strong effects of low level exposure to environmental toxicants.

Although there were indications of a prolonged TTP associated with high concentrations of POP in the Inuit population, the Inuit women had shorter TTP than both Warsaw and Kharkiv women (using the Swedish fishermen’s wives as references), who were less exposed. We can think of two possible explanations for this phenomenon. Firstly, the findings in the Inuit population may be indications of a short TTP among low exposed women rather than a prolonged TTP among highly exposed women. It has previously been suggested that the negative effect of consumption of POP-contaminated seafood may be outweighed by the positive effects of other constituents in this type of food, such as polyunsaturated fatty acids (Mendola et al., 1995; Axmon et al., 2002). However, it is unclear at which level, if any, the positive and negative effects balance each other out. A second explanation could be the fact that the number of contraceptive failures was much higher in the Kharkiv and Warsaw populations. Since a TTP cannot be established for unplanned pregnancies, a consequence is that the most fertile couples in these populations are excluded from the analyses. Thus, the results may be biased by a selection of less fertile couples.

The data which form the base for the present study have been previously analysed, using cohort (country) affiliation as a possible predictor for TTP (Toft et al., 2005). We found indications that serum concentration of p,p’-DDE, but not CB-153, was associated with fertility, a conclusion based upon the average serum concentrations of the couples in the four different countries. However, bias may have been introduced due to the differences in contraceptive use and contraceptive failures. Nevertheless, the collected evidence points towards a reduced fertility associated with higher concentrations of p,p’-DDE.

Swedish fishermen’s wives have been studied previously with respect to the TTP of their first planned pregnancy (Axmon et al., 2000, 2004b). In these questionnaire studies, no clear-cut effects were found for high fish consumption or when using CB-153 as a biomarker. Although the pregnancy of interest in the present study was each woman’s latest planned pregnancy, it is not surprising that the results from the studies are in agreement, since some 80% of the women in the present study were also included in the previous studies.

Studies performed on other populations whose main source of POP exposure is consumption of contaminated fatty fish are inconclusive for prolonged TTP, with some findings indicating a possible association between exposure and outcome (Buck et al., 2000; McGuinness et al., 2001), whereas others have failed to replicate these results (Buck et al., 1997, 1999; Axmon et al., 2000, 2001, 2004b). A possible explanation for the discrepancies in results may be the variety of measures of exposure. Several proxies of POP exposure, such as average daily intake of fish, total years of fish consumption, and assumed fish consumption during childhood and adolescence, have been used, but also biomarkers for exposure. With respect to DDT exposure, spouses of male DDT applicators have been found to have impaired fecundity (Cocco et al., 2005).

Several male and female factors must work together for a pregnancy to be achieved. If any such factor fails to work, the result may be a prolonged TTP or even no pregnancy at all. However, if a specific exposure is suspected to prolong TTP, it is not possible in epidemiological studies to establish the exact pathway from exposure to outcome (i.e. a prolonged TTP). Nevertheless, with respect to POP, previous studies indicate that male factors such as sperm motility (Rignell-Hydbom et al., 2004) and sperm chromatin integrity (Rignell-Hydbom et al., 2005) may be affected by exposure. Moreover, there are indications of menstrual cycle disturbances among women (Mendola et al., 1997; Yu et al., 2000; Eskenazi et al., 2002; Axmon et al., 2004a). Thus, the findings of prolonged TTP among the Inuit women as well as the Inuit men are biologically plausible.

To the best of our knowledge, this is the first study to use biomarkers for POP collected during the pregnancy when
assessing a possible association between POP exposure and TTP. Furthermore, even though there are some previous studies which have investigated the relation between exposure to POP and TTP, only a few have included male as well as female exposure to the compounds. In the present study we were able to investigate both male and female exposure, and because the correlation between these exposures was quite low, it was also possible to attempt to distinguish the male contribution from the female.

In conclusion, the main finding of this study was that in the Inuit population, there may be a prolonged TTP associated with serum concentrations of CB-153 and p,p′-DDE, mainly for female exposure. Due to high correlations between serum concentrations of CB-153 and p,p′-DDE for both male and female, it was not possible to determine whether the risk was associated with CB-153 or p,p′-DDE, or an interaction between the two compounds. However, previous studies indicated that the effect is most likely from serum concentrations of p,p′-DDE. The overall results of the present study create a somewhat ambiguous pattern, but give some support to the idea that dietary POP exposure might be harmful for couple fertility.

Acknowledgements

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