Fertility in women with a high intake of fish contaminated with persistent organochlorine compounds

Licentiate thesis

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This thesis is based upon the following three papers, which are included at the end and referred
to in the text according to their roman numerals.

I. Axmon A, Rylander L, Strömberg U, Hagmar L. Time to pregnancy and infertility in women with a high intake
of fish contaminated with persistent organochlorine compounds. Scand Jour Work Environ Health
2000;00:000-000.

II. Axmon A, Rylander L, Strömberg U, Hagmar L. Miscarriages and stillbirths in women with a high intake of fish

III. Axmon A, Rylander L, Strömberg U, Dyremark E, Hagmar L. Polychlorinated biphenyls in blood plasma
among Swedish female fish consumers in relation to time to pregnancy. In manuscript.
Abbreviations

CB-153 2,2’,4,4’,5,5’-hexachlorobiphenyl
CI Confidence interval
DDT Dichloro-dibiphenyl-trichloro-ethane
NYSAC New York State Angler Cohort
OH-PCB Hydroxymetabolite of PCB
OR Odds ratio
PCB Polychlorinated biphenyls
PCDD Polychlorinated dibenzo-p-dioxins
PCDF Polychlorinated dibenzofurans
POC Persistent organochlorine compounds
RBP Retinol binding protein
SuRR Success rate ratio
TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin
TTP Time to pregnancy
TTR Thyroid transport protein
Introduction

Persistent Organochlorine Compounds

Persistent organochlorine compounds (POC) can be divided into two groups; those that are deliberately manufactured, e.g. polychlorinated biphenyls (PCB) and dichloro-diphenyl-trichloro-ethane (DDT), and those that appear as by-products and pollutants to other chemical substances, e.g. polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). It has been shown that the PCB contribution to “dioxin-like” effects among high consumers of fatty fish from the Baltic Sea was almost 80%, whereas that from PCDD and PCDF was only 20% [3].

When chlorinated, PCB achieve physical properties such as chemical stability and fire resistance that have made them attractive chemicals for industrial use, e.g. in capacitors and transformers. Hence, PCB have had a widespread industrial use since they were first manufactured in 1929, with a peak around 1970. The downside is that chlorinating the biphenyls turns them into lipophilic and poorly metabolised compounds that tend to concentrate in fatty tissue. Furthermore, they are transported through the food chain, leading to dietary exposure in humans. Several PCB have been shown to cause toxic responses similar to those caused by 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) [38]. These toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproduction, development, and endocrine functions. In Sweden, the use of PCB was legislated against in 1972.

The PCB congener 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) has been found to correlate strongly with total PCB concentration [4, 16, 40]. Furthermore, it was found to be well correlated with the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalent (TEQ) in plasma from PCB [16], as well as with the total POC derived TEQ in plasma [10]. Hence, CB-153 in plasma can be considered an appropriate exposure biomarker in studying dose-response effects of PCB, and also of the total POC.

Animal studies of PCB and reproductive outcomes

In animal studies, the primary effects of TCDD on female reproduction appear to be decreased fertility and inability to maintain a pregnancy for the full gestational period [25]. Furthermore, decreased conception rates have been observed also after exposure to PCB [7, 15]. The doses causing this effect were somewhat higher than those found to result in a lowered offspring

The general structure of polychlorinated biphenyls (PCB)
birthweight, but still in the same order of magnitude [6, 8]. With respect to reproductive effects, rodents seem to be less sensitive than non-human primates, such as the Rhesus monkey [15].

Mechanisms of PCB effects on reproductive outcomes

The receptors for the steroid and thyroid hormones constitute the steroid-hormone receptor superfamily [39]. Since the structure of the hydroxymetabolites of PCB (OH-PCB) is similar to that of steroid and thyroid hormones, OH-PCB can bind to receptors from the steroid-hormone receptor family [1]. The thyroid transport protein (TTR) normally carries thyroxin over the blood-brain barrier, and over the placenta barrier to the foetus. Binding of OH-PCB to TTR leads, among other things, to lowered levels of thyroxin in the foetus, which in turn can lead to mental as well as growth retardation. Furthermore, OH-PCB prevent TTR from interacting with retinol binding protein (RBP), resulting in the inability of RBP to perform its task of transporting vitamin A. A disturbed vitamin A level may lead to sterility and growth retardation.

The OH-PCB can also bind to the oestrogen receptors, having an agonistic effect [1]. In animal studies, PCB exposure during pregnancy has been found to lower the gender ratio (ratio of male to female infants) as well as lead to feminisation of male foetuses, and polycystic ovarian syndrome in female foetuses.

Which mechanisms that cause PCB exposure to prolong the TTP, or increase the risk of miscarriages, are still unknown. However, both effects have been seen in animal studies [26], thus warranting further studies on human populations.

Time to pregnancy and frequency of miscarriages as measures of fertility

A reproductive loss can occur at any time during the pregnancy. Baird et al [5] estimated that of 100 sexually active women who start a noncontracepting cycle, approximately 26 will achieve a clinical pregnancy. An increase in the time to pregnancy (TTP) can indicate reproductive loss at any of the stages up to the clinical detection of the pregnancy. These stages include ovulation, fertilisation, implantation, and early survival of the conceptus. Obviously, TTP is only measurable if a starting point is well defined. However, in western European countries about 60-70% of all pregnancies are planned [9].

When studying time to pregnancy, the ideal design would be a prospective study of all women in the study base. However, the feasibility of using retrospective self-reports for studying subfertility, and the validity of long-term recall of TTP has been demonstrated [18, 19].
When a pregnancy is clinically recognised, reproductive loss is measured by frequency of miscarriage, or stillbirths.

Reproductive effects from exposure to PCB through consumption of fatty fish

Fish as a food item is generally considered health beneficial. It is a good source for dietary selenium [35, 37] as well as n-3 (ω-3) polyunsaturated fatty acids [34], and it is low in saturated fats and cholesterol. However, when fish resides in waters contaminated with lipophilic substances, such as PCB, the long half-lives of these substances leads to their accumulation in the fatty tissue of the fish and the transferral to humans through the diet. Two major areas of contamination are the Great Lakes region in North America, and the Baltic Sea. These waters have a low exchange rate with other bodies of water, resulting in a slow reduction of contaminating substances.

The Great Lakes

The first studies to assess the reproductive effects of eating PCB contaminated fish were carried out in the early 1980’s and onwards, using as study population pregnant women with moderate or no consumption of Lake Michigan fish, and their infants (Michigan Maternal Infant Cohort). Results showed that PCB exposure, measured by overall fish consumption as well as cord serum PCB levels, predicted a shorter gestational period, lower birth weight and smaller head circumference [14]. However, using fish consumption during pregnancy as the measure of exposure did not replicate these results.

Since the Michigan Maternal Infant Cohort was first established several populations around the Great Lakes have been studied with respect to health effects of PCB from contaminated fish.

Some of the recent results regarding contaminated fish and reproductive outcomes before birth (e.g. TTP and miscarriages) are based on studies of a group of sport fishers and their spouses (New York State Angler Cohort; NYSAC). In this group no effect on TTP has been found for either maternal [12] (number of years of fish consumption) or paternal [11] (recent frequency of Lake Ontario sport fish meals, numbers of years eating fish, and estimated lifetime PCB exposure from fish consumption) exposure. However, significant menstrual cycle length reductions were found with consumption of more than one fishmeal per month, moderate/high estimated PCB index, and consumption of contaminated fish for 7 years or more [21]. In the same study group,
no significant increases in risk for foetal death were observed across four measures of exposure: a lifetime estimate of PCB exposure; number of years of fish consumption; kilograms of sport fish consumed in 1990-1991; and a lifetime estimate of kilograms eaten [22].

In contrast to the results from the NYSAC, a study among licensed Michigan anglers and their spouses did find an association, although modest, between sport-caught fish consumption (index of life-time fish consumption) in men and risk of conception delay [13]. However, no such association was found for the women in the study group.

**The Baltic Sea**

Consumption of fatty fish from the Baltic Sea is a major exposure route for PCB to the general Swedish population [3, 36]. In order to study the health effects of this exposure, cohorts of fishermen from the east (by the Baltic Sea, exposed cohort) and west (unexposed cohort) coast of Sweden were established [17, 35]. By linkage to the national Swedish population register and to registers at the local parish offices, wives and ex-wives of these fishermen have been identified [27]. Furthermore, linkage to the population register have also provided cohorts of sisters to the fishermen (excluding women who are already in the wives cohorts) [32]. These cohorts of women have been used to investigate several reproductive, mainly post-birth, outcomes. Information on infants born to the women in the wives cohorts during the time period 1973 through 1991 [30], and to the women in the sisters cohorts from 1973 through 1993 [32] have been collected from the Swedish Medical Birth Register. Furthermore, from a subset of the women in the east coast wives cohort, blood samples have been drawn and analysed for CB-153 [29].

Infants in the east coast cohorts (both sisters and wives) have been found to have significantly lower birth weights than those born to mothers in the west coast cohorts [30, 32]. Furthermore, case-referent studies performed within the east coast wives cohorts found an increase in risk of having an infant with a low birth weight for women who had grown up in a fishing village (a proxy measure of long-time exposure), as well as for women with a high current intake of fish from the Baltic Sea [31] and for women with a high estimated past CB-153 concentration [29].

Among the fishermen's wives, the gender ratio (ratio of male to female infants) in the east coast cohort was found to be significantly lower than in the west coast cohort [30]. However,
such an effect was not found among the fishermen’s sisters [32]. No increased risk for malformations has been found in any of the east coast cohorts [28, 32].

**Studies on TTP and miscarriages**

To date, there is only one study on the effect of consumption of fish contaminated with PCB on TTP as a continuous variable [12]. In the Michigan Angler study, the outcome of interest was conception delay, defined ever having failed to conceive after 12 months of trying [13], a less sensitive measure of reproductive disturbances than TTP. Although none of these studies found any effect of consumption of contaminated fish on TTP, results from the Lake Ontario populations are not necessarily transferable to Swedish conditions.

Monkeys have been found to show similar effects as humans when exposed to PCB, and hence studies on this species are most relevant when trying to assess effects relevant to man [26]. Studies on Rhesus monkeys have shown that the level of PCB exposure that leads to decreased conception rate is in the same order of magnitude as exposure levels resulting in a lowered birth-weight [6, 7]. Considering the rather well established effects of consumption of fatty fish contaminated with PCB on birth weight [14, 29-31], it is important to further evaluate effects on other reproductive outcomes, such as TTP and frequency of miscarriages and stillbirths.
Aims of the thesis

- To evaluate effects of POC exposure on the risk for a prolonged TTP among women with a high average intake of contaminated fatty fish from the Baltic Sea.

- To evaluate the effects of POC exposure on the risk for increased frequencies of miscarriages and stillbirths among women with a high average intake of contaminated fatty fish from the Baltic Sea.
Materials and methods

In order to perform a study on TTP in the fishermen's wives cohorts mentioned in the previous chapter, we chose to contact only those born in 1945 or later (figure 1). The reason for this was that we believed that younger women would be more likely to remember the circumstances surrounding their pregnancies, than would older women. All women in our restricted cohorts were sent self-administered questionnaires, by which information (see below) on the time before the woman's five first pregnancies was collected.

Exposure measures

In papers I and II, cohort affiliation was used as the main exposure variable, using the east coast cohort as the exposed, and the west coast cohort as unexposed. Secondly, within the east coast cohort, growing up in a fishing village was used as a measure of long-time exposure, i.e. during childhood and adolescence. Thirdly, each woman within the east coast cohort was categorised as having no, medium, or high current fatty fish consumption. In the analyses women with no fatty fish consumption was regarded as the unexposed group and was used for comparisons with the high consumption, i.e. the exposed, group. The medium group overlapped the other groups and was therefore not used in the analyses.

In a previous study assessing the relation between serum CB-153 concentration and birth weight [29], blood samples had been drawn from a selected sample of the women in the east coast cohort who had given birth at least once during the time period 1973 through 1991. Data on TTP and miscarriages for 121 of these 192 women were available from the questionnaires. Information from the questionnaires was combined with CB-153 data, and CB-153 concentrations from 1995 were used to estimate the concentration at the time immediately before the pregnancy. The estimation of past CB-153 concentration took into account the reduction of body burden due to lactation (20 or 30%, depending on whether the woman had lactated for less or more than 6 months), the reduction of PCB in Baltic Sea fish over time (3% yearly decrease), as well as the biological half-life of CB-153 (5 years). For 20 of the 121 women we had access to serum obtained from a rubella screening program among pregnant women during the time period 1975-1991. CB-153 analyses of these samples were used for validation of the model for retrospective estimation. The estimated past CB-153 concentrations were then used as a fourth exposure measure (paper III).
The questionnaire focused on the women’s first five pregnancies. However, for analyses, only each woman’s first planned pregnancy was used. Choosing only one pregnancy from each woman was done in order to avoid interference from correlation between outcomes of succeeding pregnancies. The first planned pregnancy was chosen since a TTP can not be given for unplanned pregnancies. Besides TTP, the outcome of all pregnancies was recorded, and in those cases where the pregnancy ended in a miscarriage or a stillborn infant, the gestational week of this occurrence was recorded.

Figure 1. Flowchart of the women included in the different steps of the analyses.

*Time to pregnancy and infertility in women with a high intake of fish contaminated with persistent organochlorine compounds
*Miscarriages and stillbirths in women with a high intake of fish contaminated with persistent organochlorine compounds
*Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to time to pregnancy
was inquired for. The frequency of miscarriages and stillborn infants for the first planned pregnancy was used as an outcome variable along side TTP.

A woman was defined as subfertile if she reported to at any time having tried to conceive for a period of 12 months without succeeding, or if she reported a TTP of 12 months or longer for at least one of her five first pregnancies. Subfertile women who reported not to have any children were defined as infertile.

Statistics

For the statistical analysis of TTP, Cox regression was used. For each exposure group the success (i.e. pregnancy) rate was considered, and the success rate ratio (SuRR) with 95% confidence interval (CI) was calculated in order to compare two exposure groups. TTP was always right censored at 12 months in order to avoid interference from medical treatment for infertility. To graphically display the success rate in each group, Kaplan-Meier curves were drawn. These curves were also used to visually ensure that the proportional hazard assumption required by the Cox regression was satisfactory fulfilled.

To evaluate the effect of exposure on frequency of miscarriages, subfertility and infertility, odds ratios (OR) with 95% CI was calculated using unconditional logistic regression. Concentrations of CB-153 among women with and without miscarriage/stillbirth were compared using the Mann-Whitney non-parametric test.
Results

TIME TO PREGNANCY

The results of the TTP cohort study (paper I) indicated a prolonged time to first planned pregnancy for the east compared to the west coast cohort (table 1). However, this increase was found only among heavy smokers (≥10 cigarettes/day), for whom the median TTP for the east coast women was 4 months compared to 2 months in the west coast cohort (SuRR 0.68; 95% CI [0.51-0.91]; figure 2). The median TTP for the non/light smokers was 2 months for both the east and west coast cohorts (0.95 [0.81-1.12]).

Table 1. Time to pregnancy and success rate ratios (SuRR) with 95% confidence intervals (CI) for different exposure measures.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>West Median</th>
<th>East Median</th>
<th>Time to pregnancy Range</th>
<th>SuRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort affiliation</td>
<td>2.0</td>
<td>2.5</td>
<td>0-132</td>
<td>1.00</td>
</tr>
<tr>
<td>Fishing village</td>
<td>3.0</td>
<td>2.0</td>
<td>0-120</td>
<td>1.00</td>
</tr>
<tr>
<td>Fish consumption</td>
<td>2.0</td>
<td>2.0</td>
<td>0-76</td>
<td>1.00</td>
</tr>
<tr>
<td>CB-153</td>
<td>2.0</td>
<td>2.0</td>
<td>0-48</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Within the east coast cohort only 

Internal analyses within the east coast cohort were also performed (paper I), using growing up in a fishing village and current fish consumption as proxy measures for exposure. None of these measures gave any support to the hypothesis of PCB exposure leading to a prolonged TTP.

Figure 2. Time to first planned pregnancy in cohorts of east (solid line) and west (dotted line) coast fishermen’s wives stratified on smoking habits: Non/light smokers (left) and heavy smokers (right).
For the 121 women included in the biomarker study, no association was found between estimated past level of plasma CB-153 concentration and TTP (paper III).

**Subfertility and Infertility**

A slight increase in the frequency of subfertility was found among the east coast compared to the west coast women (table 3; I). As with TTP, the negative effect of east coast cohort affiliation was present only among the heavy smokers. When using fish consumption and growing up in a fishing village as proxy measures within the east coast cohort a similar pattern was seen with negative effects of exposure, although not statistically significant, apparent only among heavy smokers.

Table 2. Subfertility rates in different exposure groups, with odds ratios (OR) and 95% confidence intervals (CI).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>All women n (%)</th>
<th>Non/light smokers n (%)</th>
<th>Heavy smokers n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort affiliation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>West Coast</td>
<td>269 (36)</td>
<td>226 (36)</td>
<td>39 (34)</td>
</tr>
<tr>
<td>East Coast</td>
<td>133 (39)</td>
<td>82 (33)</td>
<td>49 (54)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.15 (0.88-1.50)</td>
<td>0.89 (0.65-1.21)</td>
<td>2.36 (1.34-4.16)</td>
</tr>
<tr>
<td>Fishing village</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>114 (40)</td>
<td>72 (34)</td>
<td>40 (52)</td>
</tr>
<tr>
<td>Yes</td>
<td>17 (39)</td>
<td>9 (28)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.96 (0.50-1.84)</td>
<td>0.74 (0.33-1.69)</td>
<td>1.85 (0.51-6.66)</td>
</tr>
<tr>
<td>Fish consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>20 (36)</td>
<td>15 (36)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>High</td>
<td>58 (39)</td>
<td>34 (31)</td>
<td>23 (61)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.15 (0.61-2.17)</td>
<td>0.81 (0.38-1.70)</td>
<td>2.76 (0.77-9.85)</td>
</tr>
</tbody>
</table>

a Within the east coast cohort only
b Information on smoking habits missing for three west coast women
c At least two meals per week
d Up to 10, and more than 10 cigarettes per day

The number of women defined as infertile was very low (11 and 10 subjects in the east and west coast cohorts, respectively). In spite of this, a statistically significant increase in infertility was found for the east coast cohort (OR 2.49 [1.05-5.92]). No stratification on smoking habits was done due to the low number of infertile women.

**Miscarriages**

In the cohort study investigating frequency of miscarriages and stillbirths (paper II), no increase in risk was found for the east coast cohort compared to the west coast cohort. This was true for all miscarriages (OR 0.69; 95% CI [0.44-1.07]) and for miscarriages stratified by gestational age (table 2). Stratification on smoking habits changed the effect estimate only marginally (data
Simultaneous stratification on smoking habits and gestational age was not possible, since the number of women with miscarriages was too low.

There was no increase in risk within the east coast cohort when current fish consumption was used as a proxy measure (OR 0.62 [0.23, 1.66]). None of the women who had grown up in a fishing village had experienced a miscarriage, and hence no analysis was performed using this exposure variable.

### Table 3. Frequency of miscarriages and stillborn infants in the east and west coast cohort, with odds ratios (OR) and 95% confidence intervals (CI).

<table>
<thead>
<tr>
<th></th>
<th>Before week 12 n (%)</th>
<th>Week 12-28 n (%)</th>
<th>After week 28 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Coast</td>
<td>12 (3)</td>
<td>11 (3)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>West Coast</td>
<td>54 (5)</td>
<td>27 (3)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>0.48 (0.26-0.92)</td>
<td>0.89 (0.44-1.80)</td>
<td>1.36 (0.44-4.19)</td>
</tr>
</tbody>
</table>

A comparison between women with and without miscarriages and stillbirths was carried out with respect to CB-153 concentrations (paper II). No attempt was made at the time to estimate past CB-153 concentration, as was done for the TTP analysis (paper III). Since this analyses was performed, however, an additional 24 CB-153 concentrations have been added to the material, and past concentrations have been estimated as described in Materials and Methods. However, as in paper III, no differences in estimated (Mann-Whitney p=0.4) and measured (p=0.8) CB-153 concentrations was found between women who had and had not experienced a miscarriage or a stillbirth (figure 3).

![Figure 3. Box-and-whiskers-plot of estimated (left) and measured (right) CB-153 lipid adjusted concentrations for east coast cohort women with and without a miscarriage or stillbirth](image)

| *Summary plot based on the median, quartiles, and extreme values. The box represents the interquartile range which contains the 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median.**
Discussion

The results indicate an increase in TTP for the east coast cohort compared to the west coast cohort, although restricted to heavy smokers (paper I). The analyses within the east coast cohort did not show that growing up in a fishing village or high current fish consumption increased TTP (paper I), neither did the use of a biomarker support any dose-response relation between PCB exposure and increased TTP (paper III).

There was no support for an increased risk for miscarriages or stillbirths for the east coast women (paper II). In fact, when considering cohort affiliation as a proxy for exposure, a decrease in early miscarriages was found. However, for late miscarriages, the results were inconclusive (reflected by a wide confidence interval) due to small numbers. Again, analyses within the east coast cohort using current fish consumption as a proxy measure did not show any increase in risk for highly exposed women, neither did the use of the biomarker CB-153 indicate any dose-response effect of PCB exposure.

If the west coast women tended to be aware of their pregnancies earlier than the east coast women, the phenomena of an increase in TTP and a decreased risk for early miscarriages would be explained. However, including only those pregnancies that ended in a live birth (90 and 87% of all pregnancies in the east and west coast cohort, respectively) in the Cox regression changed the effect estimates only marginally for the total cohort comparison (SU RR 0.88 [0.76-1.02]) as well as for light (SU RR 0.95 [0.80-1.13]) and heavy smokers (SU RR 0.72 [0.53-0.98]).

The three studies which constitute the basis for this thesis, are all based on self-administered questionnaires. With these questionnaires we sent an introductory letter informing the women about the aim of the study. It was clearly stated that we were investigating TTP as a result of intake of PCB-contaminated fish, but it was not specified that the PCB concentration was higher in fatty fish from the Baltic Sea. In a study carried out the year before, only 5% of the women from the east coast cohort were aware of potential health hazards of PCB-contaminated fish from the Baltic Sea[31]. It is therefore believed that possible recall bias in the comparison between the cohorts is of minor concern.

It has previously been shown that the socio-economic status varies little between the fishermen’s wives on the east and west coast [30]. Also, the east and west coast cohorts showed similar distributions for education and working hours (papers I, II & III). Thus, the possible confounding effect of socio-economic status was reduced by the use of the west coast cohort for comparison.
The questionnaire used was designed to study TTP, and for some factors it was therefore focused on exposure immediately before conception. The more relevant time period for the analyses of miscarriages and stillbirth is obviously early pregnancy, and some of the employed exposure measures must therefore be considered as proxies in these analyses. Some of the potential confounders were not included in our analysis since they did not differ between cohorts or fish consumption groups (coffee consumption and shift work). Other potential confounders were included in multivariate analysis since they did differ between cohorts or fish consumption groups (smoking, heavy lifts and employment status). We have no reason to assume that cohort affiliation or fish consumption affected the proportion of women who changed life-style or work habits during early pregnancy. Thus, the miscarriages and stillbirths effect estimates of cohort affiliation and fish consumption group would still be properly adjusted for these confounders.

The major weakness of the studies lies in the low response rate. However, the non-responder analysis presented in paper I indicated that this has not introduced selection bias with respect to reproductive outcome, age or smoking habits. Furthermore, in Sweden, approximately 10% of all confirmed pregnancies end in a miscarriage [20]. This is a slightly higher figure than we observed for the first planned pregnancies, but the proportion of miscarriages (up to week 28) increases to 8.7% among the fishermen's wives when considering each woman's five first pregnancies. (The discrepancy between miscarriage rate for the first planned pregnancies and all pregnancies can be explained by that women with repeated miscarriages are over-represented among those with a high parity.) It is therefore concluded that there seems to be no selection bias with respect to women responding to the questionnaire.

Women were excluded from the biomarker-study (paper III) if they had missing information on either questionnaire data (TTP), or if they lacked measured CB-153 concentration. Those excluded due to lack of CB-153 concentrations had a higher median TTP, and were older than those included. Since PCB concentrations in the Baltic Sea, as well as the consumption of locally caught fish has been decreasing since the early 1970's [30], it is reasonable to assume that these excluded women in fact had higher CB-153 concentrations than the women included. Indeed, the women excluded due to lack of information on TTP were older and had higher measured CB-153 concentrations. Hence, there might be an effect on TTP for highly exposed women not covered in the biomarker-study.

To the best of our knowledge, we are the first to use a biomarker for PCB exposure with respect to TTP and miscarriages (papers II & III). Still, a weakness of our use of the biomarker values is that they are based on estimations of past CB-153 concentrations. Our choice of parameter values for this modelling was in agreement with previous knowledge, but it should be borne in
mind that the relevant literature is sparse and inconsistent. However, when comparing the rubella validation data to the CB-153 concentrations measured in 1995 and the estimations of past concentrations calculated by our model, the estimation yielded a better agreement than the measured data. Hence, even though our model in part is dubious, the conclusion must be that it is to be preferred to the measured concentrations when focusing on critical concentrations of CB-153.

The estimated exposure to PCB through fish consumption have been found to be comparable between groups of high consumers from the Baltic Sea and the Lake Ontario regions [2]. Hence, results similar to ours should be expected from studies on populations from these areas. This holds true for risk of miscarriage, where neither we nor the NYSAC study group [22] found any effect of PCB exposure through fish consumption. However, concerning TTP, the results differ. Whereas we found an increase in TTP for heavy-smoking exposed women, neither the NYSAC group [12] nor the Michigan group [13] found any such effect. It should here be born in mind that few of the above mentioned studies use identical measures of exposure.

Several studies have found a positive correlation between consumption of non-contaminated fish during pregnancy on one hand and birth weight and duration of pregnancy on the other hand [23, 24]. As our main exposure variable we have used cohort affiliation, comparing women with an average high intake of contaminated fatty fish to women with a similar sized intake of non-contaminated fatty fish. To the best of our knowledge, our studies are the only ones to use women with a high consumption of fish as a control group, and hence the only ones to adjust for the beneficial effects of fish consumption.

For the east coast cohort, growing up in a fishing village was used as a proxy measure of high consumption of fatty fish from the Baltic Sea during childhood and adolescence. The results did not support our hypothesis of a prolonged TTP among women with this childhood exposure (paper I). On the contrary, a tendency for a beneficial effect from the assumed hazardous exposure was seen. If true, a possible interpretation of the contrasting results of an adverse effect of being a fisherman's spouse from the east coast and the apparent beneficial effect from growing up in an east coast fishing village may be that some constituents in fatty fish (e.g. polyunsaturated fatty acids) might be beneficial for germ cell development during foetal life whereas some other constituents (e.g. PCB) might be adverse to fertility during adult life.
General conclusions

**TTP, SUBFERTILITY AND INFERTILITY**

An increase in TTP for the exposed east coast cohort compared to the unexposed west coast cohort was indicated, however only for women who were classified as heavy smokers (≥ 10 cigarettes/day). Studies performed within the exposed cohort and using growing up in a fishing village and current fish consumption as proxy measures for exposure did not support the results from the cohort study. Furthermore, when using a biomarker (the PCB congener CB-153) as measure of exposure, no dose-response relation was found between exposure to PCB and TTP.

The rate of infertile women was higher in the east than in the west coast cohort, as was the rate of subfertile women. As with TTP, the negative effect of east coast cohort affiliation on subfertility was stronger among the heavy smokers.

**MISCARRIAGES AND STILLBIRTHS**

East coast cohort affiliation did not show any increased risk for miscarriages or stillbirths. The results of the internal comparisons within the east coast cohort, using current fish consumption and CB-153 as measures of exposure, gave similar results as the cohort comparison.
Further research

We have previously used growing up in a fishing village as a proxy measure for exposure to contaminated fatty fish from the Baltic Sea. In Sweden, the fisherman trade is an occupation that to a large extent is handed down from father to son. This means that large proportions of the fishermen have themselves grown up in a fisher’s family and hence have been dietary exposed to PCB since childhood. In most cases, this holds true also for their siblings [32]. Thus, a cohort of fishermen’s sisters seems an appropriate study population for further investigations regarding PCB exposure through consumption of fatty fish from the Baltic Sea and its effect on reproductive outcomes. Also, in Sweden it is not uncommon for a couple to start a family before getting married. In 1990, 13% of all children under age 18 lived with cohabiting, but not legally wed, parents, and 47% of all live births were to unmarried mothers [33]. By not restricting our cohorts to married women we therefore get a group of women more representative of the general Swedish population. Moreover, the variation in age is larger, since we also include women who plan to get married, but have not yet done so.

Only a few studies have investigated the effect of paternal exposure to PCB through consumption of contaminated fish on reproductive outcomes such as TTP and miscarriages. Furthermore, the results from these studies are not consistent. A study on licensed Michigan anglers found a modest association between sport caught fish consumption and risk of conception delay [13], whereas paternal Lake Ontario fish consumption (NYSAC) did not show any increase in risk [11]. A study on semen quality among fishermen from the Swedish east coast is currently underway. Fishermen whose wives participated in the TTP cohort study will be able to constitute a basis for a study investigating the effect of paternal exposure on TTP and miscarriages.
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