Reproductive outcomes in the Swedish Fishermen's Families Cohorts – A review

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Abstract

Persistent organochlorine pollutants (POPs) have been released in the environment for many decades, and even though the uses of many of them today are banned or restricted, they are still present in the environment. In Sweden, a major exposure route for POPs in the general population is through the consumption of fatty fish caught in the Baltic Sea, i.e. off the east coast of the country, whereas the contamination of fish from the west coast is considerably less. East as well as West coast fishermen from Sweden and their families have been found to have a relatively high consumption of locally caught fish. Consequently, these cohorts have been used to study health outcomes related to dietary exposure to POPs. In this review we bring together and summarize the findings of our previously published studies regarding reproductive health among the Swedish Fishermen's Families Cohorts.

Introduction

The term “persistent organochlorine pollutants” (POPs) is often used to include several compounds, such as polychlorinated biphenyls (PCBs), dioxins and organochlorine insecticides such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT). Although they have had different applications in the industry, POPs have several traits in common: They are lipophilic and poorly metabolized, and they bioaccumulate. While they are not very soluble in water, they have spread widely through the atmosphere, and are found in parts of the world where they were never used [1]. Moreover, even after their use has been banned, POPs are still found in human and animal tissue and in body fluids [2-6].

Concerns regarding health effects of POPs were first expressed in the 1960’s when exposed animals were found to be affected, and exhibiting thinning eggshells, morphologic abnormalities, and impaired viability of offspring [7]. Since then, the topic has been thoroughly investigated in experimental animal studies as well as epidemiological animal and human studies. Several POPs are endocrine disruptors, *i.e.* chemicals with sex steroid mimicking effects which may cause developmental and reproductive abnormalities in humans and animals [8-10].

The most common exposure route for the majority of individuals is through the consumption of contaminated foods. In Sweden, the major exposure source is consumption of fatty fish from the Baltic Sea, off the east coast of the country. Indeed, plasma levels of several of the compounds were higher in men with a high intake of such fish than in those who consumed only moderate amounts, and the levels were higher in those who ate moderate amounts of fish than in those who ate none [11].

It has been found that both professional east coast fishermen and their wives have a higher intake of Baltic Sea fish than referents from the same area [12, 13]. Similarly, on the west coast of Sweden, fishermen and their wives have a higher consumption of locally caught fish than referents from the same area. The average amount of locally caught fish consumed by fishermen and their wives on the east and west coast, respectively, has been found to be similar. However, the fish caught off the west coast of Sweden has for the past decades been much less contaminated than that caught from the Baltic Sea [14]. Thus, east coast fishermen have been found to have higher levels of POPs than both west coast fishermen and referents [12, 15]. Consequently, the east coast fishermen and their wives constitute a suitable exposed group for investigating health effects of adult dietary exposure to POPs, whereas their counterparts on the west coast is an appropriate referent group. Moreover, east coast fishermen’s sisters have been found to be more likely to have grown up in a fishing village and/or fisherman’s family [16], suggesting that they may be a fitting group when investigating health effects due to a high dietary exposure to POPs during childhood and adolescence, or even in utero. Indeed, the cohorts of fishermen, their wives and sisters, from the two coastal areas of Sweden (The Swedish Fishermen’s Families Cohorts) have over the last two decades been used to study a variety of health effects. The aim of the present paper is to describe and summarize the results found on reproductive parameters among both males and females (Table 1).

Materials and methods

Fishermen

Cohorts

In 1988, two cohorts of fishermen were established (original cohorts) [12, 17]. Records of name, date of birth, address, and date of start and end of membership were obtained from Fishermen's Organizations covering the east (Baltic Sea) and west (Kattegatt and Skagerrak) coasts of Sweden. From the east coast, information was available for fishermen who had become members during the period 1935 through 1988, although members who had left the organization before 1968 were not included. The west coast organizations provided information on members during the period 1930 through 1988, with complete listings (i.e. including those who had left the organization) from 1965 and onwards. Cohort affiliation was determined by the particular organization the fisherman was associated with. All in all, 2907 east coast fishermen (whereof 24 were females) and 8493 west coast fishermen (whereof 16 were females) were included in the cohorts.

Collecting information

In 2000, a self-administered questionnaire was sent to the 2096 east and 4854 west coast fishermen in the original cohorts who were alive as of December 31, 1999 [18]. The questionnaire was mainly aimed at investigating a possible association between dietary PCB-exposure and osteoporosis. However, it also contained a question to determine if the men would be interested in more information on a study of male reproductive function. Among the 848 east and 1766 west coast fishermen who responded to the question concerning further studies, 171 east and 308 west coast men wanted more information. In the end, 96 east and 99 west coast fishermen participated in the semen study [15, 19-21]. Information on lifestyle, and medical and reproductive history was collected through telephone interviews. Semen and blood samples were collected at the participant's residence during 2001 and 2002.

Fishermen's wives and their children

Cohorts

The original fishermen cohorts were linked to the national Swedish Population Register and to registers at the local parish offices [22]. A restriction was made to the time period for which the listings from the Fishermen's Organizations were complete, i.e. from 1968 on the east coast and from 1965 on the west coast. Thus, 2175 east and 7062 west coast women who were, or had been, married to a man in one of the original fishermen cohorts were identified. Cohort affiliation was determined by that of the fisherman to whom the woman was associated.

Of the women in the original cohorts, 1568 east and 4027 west coast women were born in 1923, or later. In 1993, these were linked to the Swedish Medical Birth Registry (MBR) [13], which includes information on almost all children born in Sweden since 1973 [23]. During 1973 through 1991, 757 women from the east coast fishermen's wives cohort gave birth to 1501 infants [13]. The corresponding numbers for the west coast were 1834 and 3553, respectively. Since the fishermen's wives cohort consisted of women who were, *or had been*, married to Swedish fishermen, the infants born are not necessarily children of the fishermen. The children were assigned the same cohort affiliation as their mothers.

Collecting information

Information on the children to the fishermen's wives was obtained through linkage to the MBR [13]. The data available thus contained information on variables such as birth weight, gestational length, parity, and pre-pregnancy weight and height of the mother. For infants born after 1982, smoking habits of the mother in early pregnancy was also recorded. Additional information was collected from the Swedish Registry of Congenital Malformations [24].

Using the information from the MBR, 72 east coast women who had given birth to a low birth-weight infant (1500-2750 g) were selected as cases in a case-control-study, using 162 east coast women with normal birth-weight infants (3250-4500 g) as controls [25]. The participating women were telephone interviewed during May and June 1994. Information was collected on fish consumption and having grown up in a fisherman's family and/or fishing village. In addition, questions were asked on dietary habits, smoking and level of education. From June to November 1995, blood samples were collected from the 57 case and 135 control mothers who could be located and was willing to supply blood [26]. In connection to this, face-to-face interviews were performed, when information was collected on estimated consumption of fatty fish from the Baltic Sea.

In 1997, a self-administered questionnaire was sent to all fishermen's wives who were born in 1945, or later [27]. For each woman's five first pregnancies, information was collected on the use of contraceptives prior to attempting to become pregnant, time to pregnancy for those pregnancies that were planned, and pregnancy outcome. Furthermore, data on several life-style and occupational factors were collected for the woman as well as her partner. The women were also asked whether they had grown up in a fishing village and/or a fisherman's family, and what their current consumption of fish was. The questionnaire was returned by 505 east and 1090 west coast women.

Fishermen’s sisters and their children

Cohorts

In 1996, the cohorts of fishermen were linked to the National Swedish Population Register in order to identify sisters (at least one parent in common) to these men [16]. Women who were included in the fishermen’s wives cohort were excluded. The cohort affiliation was determined by that of the fisherman to whom the woman was associated. The cohorts of sisters were then linked to the MBR. Thus, 1719 infants born between 1973 and 1993 to 1030 women from the east coast, and 2682 infants born in the same time period to 1537 women from the west coast were identified.

The linkages were performed by Statistics Sweden and the National Board of Health and Welfare, and the unique 10-digit personal identification code, which every citizen of Sweden has, was unknown to persons outside these organizations. Therefore, when a second study on the fishermen’s sisters was planned, a new linkage had to be done [28]. This time, a restriction was made to women who were born between 1945 and 1979. Again, women who were already included in a fishermen’s wives cohort were not included in a fishermen’s sister cohort. All in all, 1241 sisters to east coast fishermen, and 2023 sisters to west coast fishermen were included in the second fishermen’s sisters cohort. When asked, 246 east and 363 west coast women stated that they were willing to make their identity known to the researchers, thus enabling further studies.

Collecting information

Information on the children of the fishermen’s sisters were obtained from the MBR and the Swedish Registry of Congenital Malformation [16]. In 1999, all fishermen’s sisters in the second cohort were sent a self-administered questionnaire regarding their first planned pregnancy [28]. The questions were similar to those asked the fishermen’s wives two years prior (see above), however no information was collected on the woman's partner. The questionnaire was returned by 709 east and 1103 west coast women.

Among the 246 east coast women whose identity was known to the researchers, 203 had supplied a time to pregnancy in the questionnaire. These women were contacted and asked if they were willing to supply blood samples and answer a second questionnaire, containing more detailed information on all of their pregnancies [29]. In the end, 165 women provided blood in addition to answering the questionnaire.

Exposure measures

In the studies performed on the Swedish Fishermen's Families Cohorts, several measures of exposure have been used (c.f. Table 1). In those studies which rely on register data and/or questionnaires and telephone interviews, the main measure of exposure has been *cohort affiliation*, using the east coast cohorts as exposed cohorts and the west coast cohorts as referent cohorts. Moreover, within the east coast cohorts, *growing up in a fishing village and/or fisherman's family* has been used as a proxy measure of exposure during childhood and adolescence. In one of the earlier studies on the fishermen's wives, it was found that recall of fish consumption was poor [26]. Thus, throughout all future studies, *current fish consumption* was used as a proxy measure of exposure during the time of interest for each specific outcome (e.g. pregnancy attempt or child birth).

With respect to the majority of the reproductive outcomes investigated, *biomarkers* have also been used as measures of exposure. The main biomarker has been 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153). This congener has been found to correlate strongly with the total PCB concentration, as well as the total PCB-derived TEQ and the total POP-derived TEQ, in humans [30-33], and has therefore been considered not only a biomarker of PCBs, but of overall POPs. Moreover, the major DDT metabolite 1,1-dichloro-2,2-bis (*p*-

chlorophenyl)-ethylene (p,p'-DDE) which has anti-androgenic effect and is found in high levels in serum, has also been used. Throughout the studies, both biomarkers were used in their lipid adjusted form, i.e., the wet weight concentrations were standardized using the sum of serum concentrations of triglycerides, cholesterol and phospholipids [30].

Since many of the studies on the Swedish Fishermen's Families Cohort have been of a retrospective nature, a drawback is that the biomarkers describe current, rather than past, exposure. Therefore, a model to estimate past exposure was derived [34]. This model assumed a constant consumption of fatty fish, and a constant background exposure. However, the model took into consideration the decreasing levels of POPs in fatty fish, the reduction of body burden due to lactation, and the half-life of the compound in humans. The model was validated for CB-153 in women using bio-banked samples from a rubella screening performed years earlier. The model has since then been validated for male CB-153, as well as for both male and female p,p'-DDE [35].

Results

Exposure levels

The median plasma concentration of CB-153 among the fishermen' wives from the Swedish east coast was 160 ng/g lipid (range 16-780) and the median serum concentration among the sisters to the fishermen from the Swedish east coast was 115 ng/g lipid (range 27-560). With respect to the estimated past exposures, this varied from study to study depending on the time for which the concentrations were estimated, and which parameters were used in the model. In those studies where estimated past exposure was related to any outcome, the estimated past concentrations was between 36% and 124% higher than the measured. The median serum concentrations among the fishermen were 193 ng/g lipid (range 40-1460) for CB-153 and 240 ng/g lipid (range 40-2250) for p,p'-DDE.

Male reproductive parameters

Throughout the studies concerning male reproductive parameters, serum concentrations of CB-153 and p,p'-DDE were analyzed both as continuous measures (untransformed or log transformed) and categorized into five equally sized groups: For CB-153 the cut-points were 112, 167, 232, and 328 ng/g lipid, and for p,p'-DDE 136, 191, 273, and 471 ng/g lipid [15, 19-21]. The correlation between CB-153 and p,p'-DDE for exposure was strong (r=0.78). Thus, the two biomarkers were never used simultaneously in any model.

Sperm motility, sperm concentration, semen volume and total sperm count

Semen volume, sperm motility and sperm concentration were analyzed according to the Guidelines of the World Health Organization (WHO) [36]. The possible effects of CB-153 and p,p'-DDE on these outcomes were investigated using linear regression. The biomarkers of exposure were entered into the model either as continuous variables (as measured or using the natural logarithm transformation) or as dummy variables in their categorized form [15].

A negative association, although not statistically significant when age was taken into account, between serum concentrations of CB-153 and the proportion of motile sperm was found, in that an increase in CB-153 of 100 ng/g lipid implied a decrease of motile sperms of 2% (95% confidence interval [CI] 0.5, 3.6; age adjusted 1.4%, CI -0.3, 3.1) [15]. Moreover, the subjects in the quintile with the highest CB-153 concentrations had lower sperm motility compared to the subjects in the lowest exposed quintile (mean difference 14 %, 95% CI 4.5, 23; age adjusted 9.9%, 95% CI -1.0, 21). Similar results were found for p,p'-DDE, where an increase by 100 ng/g lipid was associated with a mean decrease of motile sperms of 1.0% (95% CI 0.1, 2.0; age adjusted 0.6 %, 95% CI –0.4, 1.7).

No consistent associations were found between any of the two biomarkers and semen volume, sperm concentration, total sperm count or sperm morphology [15, 37].

Sperm chromatin integrity

Sperm Chromatin Structure Assay (SCSA) was used to detect DNA fragmentation (small breaks in the sperm chromosome) [38, 39]. The DNA fragmentation index (%DFI) was used to express the percentage of sperms showing DNA fragmentation, whereas the high DNA stainability (%HDS) described the percentage of sperms with incomplete chromatin condensation [20]. The possible effects of CB-153 and p,p'-DDE on %DFI and %HDS were investigated using linear regression. The biomarkers of exposure were entered into the model either as continuous variables (as measured or using the natural logarithm transformation) or as dummy variables in their categorized form. Since the distributions of %DFI and %HDS were skewed, both variables were transformed using the natural logarithm (ln) before inclusion in any models.

Men in the lowest CB-153 quintile had 41% (95% CI 11, 78) lower ln %DFI than the men with higher CB-153 concentrations [20]. The pattern was similar for p,p'-DDE, however these results were affected by the inclusion of age in the model (age adjusted 22%, 95% CI -5, 53). With respect to continuous measures of CB-153 and p,p'-DDE there were no consistent association with %DFI, neither were there any associations between the two measures of exposure and %HDS.

Y:X chromosome ratio

Two color fluorescence in situ hybridization (FISH) was used to assess sperm chromosome Y:X ratio in an ejaculate [21]. The possible effects of CB-153 and p,p'-DDE on Y:X chromosome ratio were investigated using linear regression, thereby estimating the linear regression coefficient (β). The biomarkers of exposure were entered into the model either as continuous variables (as measured or using the natural logarithm transformation) or as dummy variables in their categorized form.

Both CB-153 and p,p'-DDE were found to associate with the Y chromosome fraction [21]. For ln CB-153, β was 0.42 (95% CI 0.01, 0.83), which may be interpreted such that the Y chromosome fraction will increase by 0.04 for each 10% increase in CB-153 [40]. The corresponding numbers for ln p,p'-DDE were β = 0.66 (95% CI 0.30, 1.01) [21], which implies a 0.06 increase in the Y chromosome fraction for each 10% increase in p,p'-DDE [40]. When comparing the lowest exposed quintile to the highest, the effect was more marked for p,p'-DDE (mean difference 1.6%, 95% CI 0.8, 2.5) than for CB-153 (mean difference 0.8%, 95% CI -0.1, 1.7) [21].

Seminal levels of markers of epididymal and accessory sex gland function

Fructose, zinc, prostate specific antigen (PSA), and neutral α-glucosidase (NAG) were analyzed as markers for the function of the epididymis, prostate and seminal vesicles [19]. The possible effects of CB-153 and p,p'-DDE on these outcomes were investigated using linear regression, thereby estimating the linear regression coefficient (β). The biomarkers of exposure were entered into the model either as continuous variables (as measured or using the natural logarithm transformation) or as dummy variables in their categorized form.

Among the outcomes investigated, the strongest associations were found for total PSA [19]. For continuous CB-153, β was -2.5 (95% CI -4.0, -0.9; adjusted for age, smoking and abstinence time β = -1.4, 95% CI -3.0, 0.1), and for continuous p,p'-DDE β was -0.96 (95% CI -1.96, 0.03; adjusted for age, smoking and abstinence time β = -0.32, 95% CI -1.26, 0.63). Moreover, men in the highest quintile of CB-153 had on average 1350µg/l (95% CI 407, 2298; adjusted for age, smoking and abstinence time 556 µg/l, 95% CI -421, 1532) lower PSA compared to the men in the lowest quintile of CB-153. No such effect was found for p,p'-DDE. With respect to the other outcomes investigated, no associations were found with either biomarker of exposure.

Hormone parameters

Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol were determined using immunofluorometric techniques [15]. Moreover, serum testosterone and sexual hormone binding globuline (SHBG) were measured by commercially available immunoassays, and inhibin B levels were assessed using a specific immunometric method [41]. These hormone parameters, as well as the ratio between serum testosterone and SHBG, were all analyzed with respect to a possible association with CB-153 and p,p'-DDE, using linear regression [15]. The biomarkers of exposure were entered into the model either as continuous variables (as measured or using the natural logarithm transformation) or as dummy variables in their categorized form.

Although men in the lowest quintile with respect to CB-153 had lower SHBG (29.4 nmol/l vs. 31.5 nmol/l; p=0.04) and higher testosterone/SHBG ratio (0.48 vs. 0.43; p=0.07) than those in the highest quintile, no effects were seen when adjusting for age. No associations were found with respect to CB-153 concentrations and the other outcomes investigated, neither were any associations found between p,p'-DDE and any of the hormone parameters.

Female reproductive parameters

Age at menarche

Both the fishermen's wives and the second cohorts of fishermen's sisters were asked when they had their first menstruation [42]. Since the investigate outcome was not related to adult fish consumption, women who had grown up in a fishing village and/or fisherman's family on the east coast were considered as exposed. As referents, three groups of women were used: 1) women from the east coast who had not grown up in a fishing village and/or fisherman's family, 2) women from the west coast who had not grown up in a fishing village and/or fisherman's family, and 3) women from the west coast who had grown up in a fishing village and/or fisherman's family. Analysis of variance (ANOVA) was used to determine the possible differences between the exposed and referent groups. All analyses were adjusted for year of birth.

The results indicated that the exposed women tended to be somewhat older at menarche than referents from the same coastal area (mean age 13.0 vs. 12.8 years) [42]. However, no differences were found between the exposed women and the women from the west coast (mean age 13.0 years for all groups). The results were similar when analyzing fishermen's wives and fishermen's sisters separately, although there was a lack of statistical power due to the lower number of observations.

Menstrual cycle length

In the self-administered questionnaires, the fishermen's wives were asked about their average menstrual cycle length during periods when they were not using oral contraceptives, were pregnant, or lactating, whereas for the fishermen's sisters the question about menstrual cycle length was posed only to those who reported a planned pregnancy, and menstrual cycle length was then related to the time when they were trying to conceive [43]. The effect of the different measures of exposure on menstrual cycle length was evaluated using linear regression. Since the east coast women tended to smoke more than the west coast women, all results were adjusted for smoking habits. The east coast women had on average 0.46 (95% CI 0.03, 0.89) days shorter menstrual cycles than the west coast women. Among the fishermen's wives and fishermen's sisters in the east coast cohorts, those with a high consumption of fatty fish had on average 0.48 (95% CI -0.08, 1.05) days shorter menstrual cycles than those with no or low fish consumption. However, there were no difference between women who had and had not grown up in a fishing village and/or fisherman's family, neither was there any effect of serum/plasma concentrations of CB-153.

Miscarriages and stillbirths

Among the fishermen's sisters, data from the MBR was used to determine pregnancies which had ended in a stillbirth [16]. Odds ratios (ORs) with 95% CI were estimated using logistic regression. The analyses revealed a slightly raised, but not statistically significant, risk for stillbirth among the east coast women (OR 1.6; 95% CI 0.7, 3.4). With respect to data collected through the self-administered questionnaires distributed to fishermen's wives [44] and fishermen's sisters [28], miscarriage was determined by using information on the outcome of the first planned pregnancy (miscarriages and stillbirths). Among the fishermen's sisters, the miscarriage rate was compared to the number of live births, whereas for the fishermen's wives the comparisons were made to the number of live births, extrauterine pregnancies, and induced abortions. The analyses also differed in that for the fishermen's wives all analyses were stratified on gestational age (<12 weeks, 12-28 weeks, and >28 weeks), whereas for the fishermen's sisters, only total risk were presented. However, comparable analyses are available online [45]. In each analysis, the OR for miscarriage, with a 95% CI, was estimated using logistic regression. There were weak indications of a decreased risk for miscarriage among the east coast fishermen's wives (OR 0.68; 95 % CI 0.44, 1.06) [45] and fishermen's sisters (OR 0.86; 95% CI 0.57, 1.31) [28]. Stratifying on gestational age, the effect was most apparent among early (OR 0.48; 95% CI 0.26, 0.92) miscarriages in the fishermen's wives cohort [44]. The corresponding analyses among the fishermen's sisters did not reveal any similar effect (OR 1.03; 95% CI 0.51, 2.09 for early and OR 0.71; 95% CI 0.37, 1.35 for late miscarriages) [45], neither did the analyses for stillbirths among the fishermen's wives (OR 1.36; 95% CI 0.44, 4.19) [44]. Since none of the fishermen's sisters experienced a stillbirth, no such analysis was performed for this group.

Couple fertility (time to pregnancy)

In the self-administered questionnaire, the fishermen's wives were asked about the time to pregnancy (TTP) for their first five pregnancies [27]. The question was phrased "How many months of trying did it take you to become pregnant?". For the analyses, the first planned pregnancy was chosen. In the fishermen's sisters questionnaire, a line of questions were used to establish TTP: "Did you become pregnant the first month of trying? If no, did you become pregnant the second month of trying? If no, in which month did you become pregnant?" [28]. Furthermore, the fishermen's sisters were asked only about their first planned pregnancy. The data from the fishermen's sisters were analyzed using Cox regression, estimating a Success Rate Ratio (SuRR), whereas the fishermen's sisters data were analyzed applying logistic regression to a database of months (cycles) and estimating the fecundability odds ratio (FOR). It has been shown that these two methods are comparable [45]. However, post-publication of the fishermen's wives data, the FORs for this material were also calculated [45]. A decreased fertility (i.e. prolonged TTP) was found for the east coast fishermen's wives as compared to the west coast fishermen's wives (SuRR 0.86; 95% CI 0.75, 0.99) [27]. The effect was most apparent among women who smoked more than 10 cigarettes per day, i.e. comparing east and west coast smokers (SuRR 0.68; 95% CI 0.51, 0.91). Among the fishermen's sisters, no effect was found for cohort affiliation (FOR 0.99; 95% CI 0.87, 1.14) [28]. The effect among smokers was not apparent in the group of fishermen's sisters. Among the east coast women, growing up in fishing village and/or fisherman's family did not suggest any negative effect on TTP, neither did a high consumption of fatty fish [27, 28, 45] or serum/plasma concentrations of CB-153 [29, 45, 46].

Infant parameters

Birth weight

Data from the MBR have been used in cohort studies to investigate infant outcomes among the fishermen's wives [13] and fishermen's sisters [16]. In the study among the fishermen's wives, the birth weight distribution in the east coast cohort was compared to that in the west coast cohort using the chi-square test based on weight groups (intervals of 500 g), whereas among the fishermen's sisters, birth weight was analyzed as a continuous variable in linear regression models. Furthermore, logistic regression was used to estimate the OR for low birth weight, using 2500 g (both studies) and 3000 g (only for the fishermen's wives) as cut-off points. All analyses were performed after the exclusion of multiple births and infants with major malformations.

Among the east coast fishermen's wives, the median birth weight was found to be 3530 g, which was lower than the corresponding figure for the west coast fishermen's wives (3610 g; p<0.001) [13]. The absolute birth weight difference was slightly lower among the fishermen's sisters (3500 g for the east coast women and 3560 g for the west coast women), although the statistical testing still found it significant (p<0.001) [16]. When east coast fishermen's wives and sisters were taken together, and compared with the corresponding west coast women, and OR of 1.4 (95% CI 1.1, 1.8) was obtained using 2500 g as cut-point. The OR alternatively using 3000 g as cut-point was also 1.4 (95% CI 1.2, 1.6).

Among the east coast fishermen's wives, a case-control study was performed using 90 infants with a birth weight between 1500 g and 2750 g as cases, and 162 infants with birth weight in the interval 3250 g to 4500 g as controls [25, 34]. The effect of fish consumption and CB-153 on birth weight was considered using ORs estimated by means of logistic regression.

A high total current intake of fish from the Baltic Sea indicated an increased risk of having an infant with low birth weight (OR 1.9; 95% CI 0.9, 3.9), although no dose-response relation was found [25]. Furthermore, when analyzing estimated intake of fish during the time period of interest (i.e. when the infant was born), the effects were diminished. However, childhood exposure (measured as growing up in a fishing village) carried an increased risk of giving birth to a low birth weight infant (OR 2.1; 95% CI 1.0, 4.3). The case mothers had higher median concentrations of CB-153 than the control mothers (190 ng/g lipid versus 160 ng/g lipid) [34].

Birth length and head circumference

Information on birth length and head circumference were obtained from the MBR for the fishermen's wives [13] and the fishermen's sisters [16]. The median length was 51 cm for east and west coast fishermen's wives as well as sisters (p=0.2 for the fishermen's wives and p=0.8 for the fishermen's sisters). Among the fishermen's wives as well as the fishermen's sisters, the distribution of head circumference of the east coast infants was shifted downwards compared to the west coast infants (p<0.001 for both groups of women).

Fetal growth

Among the fishermen's sisters [16], fetal growth retardation (small for gestational age; SGA) was used as an outcome measure, using the definition given by Källén [47]. Odds ratios (ORs) for SGA were estimated using logistic regression. The analyses revealed that there was a slightly higher risk for east coast infants of being SGA (adjusted OR 1.4; 95% CI 0.9, 2.1). When east coast fishermen's wives and sisters were taken together and compared with the corresponding west coast women, an OR of 1.5 (95% CI 1.2, 2.0) was obtained [16].

Gender ratio

When gender ratio (boys to girls) was investigated for the infants born to the fishermen's wives, the chi-square test was used to compare the east coast women to the west coast women [13]. The analyses found that the gender ratio was lower in the east coast cohort (0.98 versus 1.11; p=0.05). However, neither gender ratio differed significantly from 1.06, which is the ratio for the overall Swedish population. Among the fishermen's sisters the gender ratio was in the east coast cohort 1.03 and in the west coast cohort 0.99 [16].

Malformations

Infants born to east and west coast fishermen's sisters were compared with respect to malformations [16]. ORs for malformations were calculated using logistic regression. Among the fishermen's wives comparisons were performed with expected numbers based on data from the regional populations [24].

No specific malformation was overrepresented in the east coast cohorts [16, 24].

Discussion

The Swedish Fishermen's Families Cohorts consist of a large number of men and women of varying ages. The east coast cohorts comprises individuals who have been exposed to POPs at different times in their lives; those who are born and grown up in a fisherman's family and/or a fishing village are likely to have been exposed to POPs in utero and during their childhood, whereas others may have had high exposure only during their adult life. Indeed, the men and women in the east coast cohorts have been found to have higher average levels of POPs than men and women from the general Swedish population [30, 48, 49], and those who have grown up in a fishing village and/or fishermen's family have been found to have especially high POP levels [49]. Thus, a major strength with the Swedish Fishermen's Families studies is the use of a large and relatively highly exposed study population.

The men and women in the east coast Swedish Fishermen's Families Cohorts have been found to eat more fish than individuals from the general Swedish population [13, 16, 22]. Since fish have constituents, e.g. long-chain n-3 fatty acids, minerals and vitamins, which may have positive effects on reproductive health [50-52], the general population may not be a suitable reference group in studies of health effects of dietary exposure to POPs. However, the waters off the west coast of Sweden are considerably less contaminated than the Baltic Sea [14]. The west coast Swedish Fishermen's Families Cohorts have been found to eat fatty fish at a rate comparable to that of the east coast cohorts [13, 22], but to have considerably lower levels of POPs [11]. Moreover, although the east coast fishermen's wives tend to smoke more than those in the west coast cohorts [22, 44], the west coast cohorts have been found to be similar to the east coast cohorts with respect to other socioeconomic factors, such as alcohol consumption [13, 16, 22] and educational level [16, 44]. Hence, another major strength with the Swedish Fishermen's Families studies is the use of large and socio-economically similar cohorts as referent groups.

In several of the studies performed on the Swedish Fishermen's Families Cohorts, biomarkers for exposure to POPs have been used. Most frequently, CB-153 has been considered as a biomarker for a person's total body burden of POP. The rationale for this is that this PCB congener has been found to correlate strongly with the total PCB concentration in plasma [30], serum [31], whole venous blood [53], and cord blood [53], as well as with the TEQ from total PCB [30, 33] and total POP-derived TEQ [2]. Other proxy measures of exposure used in the Swedish Fishermen's Families Cohorts, i.e. cohort affiliation, fish consumption and grown up in a fisherman's family and/or fishing village, have been found to be strongly associated with the concentrations of CB-153 [11, 30, 49]. Thus, a further strength with the Swedish Fishermen's Families Cohorts studies is the use of robust and valid proxies for exposures.

When they enter the human body, POPs are stored in the adipose tissue. Thus, to obtain a valid measure of the body burden of POP, a biopsy should be performed and fatty tissue should be analyzed. This is, however, a procedure which is more costly and painful than collecting a blood sample. Nevertheless, it may be assumed that the concentration of POP in serum lipids is in equilibrium with that in adipose tissue. Indeed, Kahn et al [54] found a very high correlation (r=0.89) between 2,3,7,8 TCDD concentration in fat biopsy and the lipid adjusted serum concentration. Thus, the body burden of POP may be estimated by calculating the lipid standardized serum concentrations. However, Schisterman et al [55] have recently shown that this method is highly prone to bias, causing an underestimation of the true effect. Nevertheless, the correlations between wet weight and lipid adjusted concentrations of POPs in the Swedish Fishermen's Families Cohorts are high [30, 49]. Moreover, the results obtained when using the biomarkers are in agreement with those obtained using the proxy measures of exposure. Thus, a possible bias introduces by the lipid standardization should pose, at most, a minor problem.

The most consistent results from the Swedish Fishermen's Families Cohorts are those on infant parameters, and especially birth weight and fetal growth. Irrespective of which measure of exposure was used, children who are exposed to POPs in utero have an increased risk of weighing less at birth, also after having taken the gestational length into account (SGA). Similar findings have been observed among fish eaters from the Lake Michigan area in the US who had PCB levels comparable to the ones among the Swedish fishermen's wives [56]. Also in the general population in the Netherlands with relatively low POP exposures, in utero levels of PCB were negatively associated with birth weight [57]. There are, however, also epidemiological examples showing no, or even positive, associations between POP exposure and birth weight [58-60]. The overall picture regarding the hypothesized association between POP exposure and birth weight is, accordingly, still far from conclusive.

Environmental chemicals acting as sex hormone agonists or antagonists could have an adverse effect on male sex hormones which in turn might decrease the male reproductive function [10, 61]. In our study sperm motility decreased with increasing serum levels of POP. This finding is consistent with four other cross-sectional studies carried out during recent years [62-65]. Sperm DNA integrity is essential for the accurate transmission of genetic information and sperm chromatin abnormalities or DNA damage may result in impaired male fertility [66]. Among Swedish fishermen we found that increasing POP exposure increased the level of sperm DNA strand breaks. However, in a study comprising Inuits, with higher serum levels of POP, the levels of sperm DNA strandbreaks were low. This inconsistent result might be explained by differences in the genetic background and lifestyle habits, which still needs to be elucidated. The mechanism behind changes in Y:X ratio is unknown. However one hypothesis could be a loss of X chromosome due to an effect on formation of micronuclei during the meiosis, by *e.g.* POPs. We found effects of POP biomarkers on the proportion of Y- and X-bearing sperm. However, such effects were not seen in three other study populations [67]. The inconsistent results might be explained by differences in POP exposure profile and dose due to variation in lifestyle and diet. Regarding sperm count and sperm morphology none of these outcomes were related to POP exposure.

The results concerning female reproductive parameters are ambiguous, and in some cases they suggest a protective, rather than hazardous, effect of fish consumption. Taking other studies on the topic into account, no obvious risk pattern emerges for most of the outcomes. Although results from a cross-sectional study on Akwesasne Mohawk girls suggested that high concentrations of blood PCB lead to a higher probability of early menarche [68], retrospective studies performed by Vasiliu et al [69] and in the Swedish Fishermen's Families Cohort studies found no such effect. Moreover, whereas exposure to POPs was associated with a shortened menstrual cycle length in the retrospective studies performed in the Swedish Fishermen's Families Cohorts and the New York State Angler Cohort [70], another retrospective study found the opposite relation between exposure and outcome [71], whereas a prospective study using daily urine sampling found no relation at all [72]. With respect to miscarriages and stillbirths, the results available are more homogenous than those for the other outcomes. In the Swedish Fishermen's Families Cohorts, none of the analyses produced a statistically significant hazard associated with POP exposure. However, in some of the subgroup analyses, POP exposure was suggested to have a protective effect. This is in agreement with other studies, were no effect [73-75], or a protective effect [76], has been found.

East coast fishermen's wives were found to have longer TTPs than west coast fishermen's wives. However, these results were not replicated among the fishermen's sisters, neither did any of the exposure measures within the east coast cohorts suggest any effect of POP exposure. In the New York State Angler Cohort, several studies have been performed regarding parental POP exposure and fertility. The majority of these results show no effect on

TTP, delayed conception, or infertility of maternal [77, 78] or paternal [79, 80] exposure, although one study did suggest reduced fecundability among women with high consumption of fatty fish from the Great Lakes [80]. In the INUENDO study, results suggested that high exposure to POPs were associated with reduced fertility only among Inuits from Greenland, and not among couples, i.e. maternal and paternal exposure, from Poland, Ukraine or Sweden [81].

When evaluating the results from the Swedish Fishermen's Families Cohorts studies, it is important to keep in mind that the main exposure source for POP, namely fatty fish from the Baltic Sea, is also an exposure route for other constituents of fish. These other constituents include both other pollutants, such as methyl mercury, and natural components, such as marine n-3 fatty acids, minerals and vitamins. In the Swedish Fishermen's Families Cohorts, the concentration of CB-153 has been found to be correlated (r_S=0.51) to that of methyl mercury [82]. However, where associations were found between on one hand CB-153 and p,p'-DDE and on the other hand several male reproductive parameters, no consistent relationships were found between methyl mercury and the same male parameters.

With respect to the natural components of fatty fish, marine n-3 fatty acids have been found to be associated with prolonged gestation in randomized trials [83-87]. Moreover, positive associations between seafood consumption and reproductive outcomes, such as birth weight and gestational length, has been seen in observational studies [51, 52, 88-92]. Thus, there is a possibility that the effect of the potentially hazardous constituents in Baltic Sea fatty fish could be outweighed by their beneficial counterparts, which would explain the positive results found for some outcomes.

Conclusions

The main finding from the studies performed on the Swedish Fishermen's Families Cohorts is that although there may be minor effects on both the male and female reproduction system, the major effects are those concerning the growth of the fetus. This observation was strengthened by the fact that the negative effect on birth weight was observed for all exposure measures used. In addition, corresponding effects have been observed in other populations with a similar exposure source, i.e. intake of contaminated fish.

Regarding male reproduction, the studies on the Swedish Fishermen's Families Cohort, as well as studies on other populations, suggest that men with higher exposure levels of POPs were more likely to have impaired sperm motility and increased percentage of sperm showing DNA fragmentation. However, the slightly higher proportion of Y-chromosome bearing sperms found in the Swedish cohorts has not been replicated in any other populations so far.

With respect to female reproductive parameters, the Swedish data together with other epidemiological studies, provide no conclusive results. It is likely that at least some of the discrepancies between studies are due to differences in study design, nor can it be ruled out that the results regarding POPs and reproductive outcomes were disturbed by competing exposures, both hazardous (e.g. methyl mercury) and beneficiary (e.g. marine n-3 fatty acids).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AA initiated the project. All authors participated in drafting the manuscript, and have approved of the final version.

Acknowledgements

The studies on the Swedish Fishermen's Families Cohort were all carried out in collaboration with Professor Lars Hagmar, who passed away in June 2006.

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