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Fertility and female dietary exposure to persistent organochlorine compounds

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LUND
UNIVERSITY

To my family

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Contents

Abbreviations

Ah	Aryl hydrocarbon
CB-153	2,2',4,4',5,5'-hexachlorobiphenyl
CI	Confidence Interval
DDE	Dichloro-diphenyl-dichloro-ethylene
DDT	Dichloro-diphenyl-trichloro-ethane
ER	Oestrogen receptor
LOAEL	Lowest Observable Adverse Effect Level
MBR	The Swedish Medical Birth Register
NOAEL	No Observable Adverse Effect Level
OR	Odds Ratio
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzo-p-dioxin
PCDF	Polychlorinated Dibenzofuran
PHDD	Polyhalogenated Dibenzo-p-dioxin
PHDF	Polyhalogenated Dibenzofuran
POC	Persistent Organochlorine Compound
SOR	Success Odds Ratio
SuRR	Success Rate Ratio
TCDD	2,3,7,8-tetrachloro-dibenzo-*p*-dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalency

List of Papers

This thesis is based upon the following five 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 J Work Environ Health 2000;26(3):199-206. *(Reproduced by permission of the Scandinavian Journal of Work, Environment and Health.)*

II. Axmon A, Rylander L, Strömberg U, Hagmar L. Miscarriages and stillbirths in women with a high intake of fish contaminated with persistent organochlorine compounds. Int Arch Occup Env Med 2000;73:204-208. *(Reproduced by permission of Springer-Verlag.)*

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. J Toxicol Environ Health A 2001;64(6):485-498. *(Reproduced by permission of Taylor & Francis, Inc, http://www.routledge.ny.com)*

IV. Axmon A, Rylander L, Strömberg U, Hagmar L. Female fertility in relation to the consumption of fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 2002;28(2):124-132. *(Reproduced by permission of the Scandinavian Journal of Work, Environment and Health.)*

V. Axmon A, Rylander L, Strömberg U, Jönsson B, Nilsson-Ehle P, Hagmar L. Polychlorinated biphenyls in serum and time to pregnancy. Submitted.

Introduction

PERSISTENT ORGANOCHLORINE COMPOUNDS

Persistent organochlorine compounds (POCs) can be divided into two groups: those that are deliberately manufactured, e.g. polychlorinated biphenyls (PCBs) 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 (PCDDs) and polychlorinated dibenzofurans (PCDFs).

The term "dioxins" refers to a large group of polyhalogenated dibenzo-*p*-dioxins (PHDDs) and dibenzofurans (PHDFs). Included in this group are the 210 polychlorinated congeners PCDD and PCDF, of which 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD; Figure 1) is the most powerful (68) and the one often referred to as "dioxin". This congener achieved notoriety when it was discovered to be a contaminant in the herbicide Agent Orange, and has been found be the most biologically active and toxic dioxin (57).

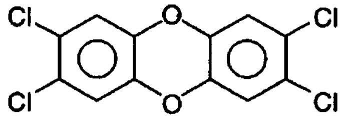


Figure 1. 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD).

PCBs (Figure 2) form a class of 209 congeners in which 1 to 10 chlorine atoms are attached to biphenyl. Congeners without chlorine in the ortho-positions (2,2',6 and 6'; non-ortho congeners) can assume a coplanar conformation which resembles TCDD (2). The physical and chemical properties of PCBs, e.g. their resistance to acids and bases, and their thermal stability and low flammability, have made the compounds attractive in several industrial applications. The commercial production of PCB mixtures started in 1929, and the chemicals were marketed under names such as Aroclor, Kanechlor and Chlophen. They were used mainly in capacitors and transformers, but also as heat transfer fluids, hydraulic fluids, lubricants, and additives in paints, plastics and dyes. In Sweden, the use of PCBs was legislated against in 1972.

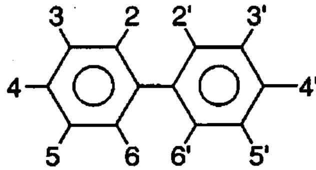


Figure 2. The general structure of polychlorinated biphenyls (PCBs).

DDT (Figure 3) was introduced in the 1930's as a pesticide and insecticide (12). In Sweden the usage peaked in the 1960's. The agricultural use was legislated against in 1970, and use of DDT in forestry was banned five years later. In other

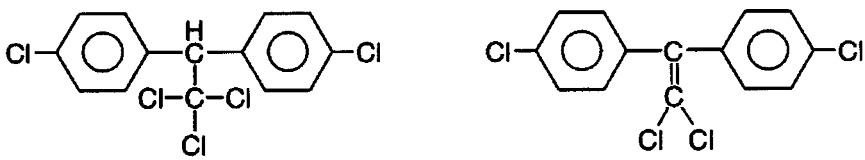


Figure 3. Dichloro-diphenyl-trichloro-ethane (DDT; left) and dichloro-diphenyl-dichloro-ethylene (DDE; right).

parts of the world, however, it is still used in malaria control. DDT is metabolised to the stable lipophilic metabolite dichloro-diphenyl-dichloro-ethylene (DDE; Figure 3).

Besides the most common POCs described above, several other organochlorine compounds have been introduced to the environment as e.g. pesticides (methoxychlor, heptachlor, and chlordane) (3). Furthermore, some of the unintentionally formed POCs are still brought into the environment. An example of this is hexachlorobenzene, which is formed in combustion processes such as uncontrolled waste incineration.

The qualities that made POCs desirable for industrial and agricultural use have also caused them to persist in the environment. Today they can be found in almost all compartments of the biosphere, including animal and human tissues and body fluids (26). Since they are lipophilic and poorly metabolised, they tend to accumulate in fatty tissue. They are transported through the food chain, leading to dietary exposure in humans. In some regions, meat and dairy products are the most important sources of dietary exposure (3). In Sweden however, one of the major sources of exposure is through the consumption of fatty fish from the Baltic Sea (3, 12, 90).

In both humans and animals, PCBs and dioxins have been found to produce a variety of effects, such as hepato-, immuno-, neuro-, dermal and reproductive toxicity as well as cancer (26). DDT has been found to be less toxic than the other compounds, but has nevertheless been shown to be a neurotoxicant in humans, and has been suggested to affect some reproductive outcomes, such as fertility and duration of lactation (1).

CB-153 AS A BIOMARKER FOR POC EXPOSURE

The toxicity of dioxins and PCB congeners is often related to the toxicity of TCDD. To simplify these comparisons, a system was introduced by which each PCB/PCDD/PCDF was assigned a toxic equivalency factor (TEF), which estimates the Ah receptor agonistic potency of the chemical to that of TCDD (81). The TEF values for individual compounds can then be used to determine toxic equivalents (TEQ) for mixtures of PCB/PCDD/PCDF.

The PCB congener 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153; Figure 4) have been found to correlate strongly with the total PCB concentration in plasma (44), serum (95), whole venous blood (45), and cord blood (45), as well as with the total PCB concentration in Baltic Sea fatty fish (13). Furthermore, it is well correlated with the TEQ from total PCB in both plasma (44) and human breast milk (41) as well as with the total POC derived TEQ in plasma (26). The correlation between lipid adjusted concentrations of CB-153 and DDE is also high (r_S=0.73; own unpublished data). Thus,

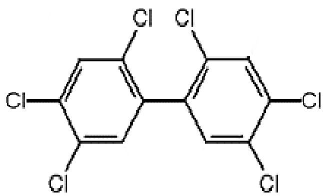


Figure 4. 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153).

CB-153 can be used as a proxy of a person's total body burden of PCB as well as POC.

A person's body burden of CB-153 depends on the intake of the congener. When a major source for this intake is considered to be Baltic Sea fatty fish, the actual intake of CB-153 is not only dependent on the intake of fish, but also on the concentration of CB-153 in the fish, which in turn is dependent on the levels of the chemical in the environment. Furthermore, the rates of metabolism and excretion of CB-153, which is reflected in the biological half-life of the compound, also affects the body burden. Finally, a woman's body burden of PCB is substantially reduced during lactation (55, 73, 74, 84).

ANIMAL STUDIES ON REPRODUCTIVE EFFECTS OF EXPOSURE TO POC

When a Medline search using the MeSH headings "Macaca Mulatta" (Rhesus monkey) and "Polychlorinated Biphenyls" was performed, only three major studies were found: Barsotti and colleagues exposed 30 female monkeys to the commercial mixture Aroclor 1248 (4, 5, 16, 24, 25, 56) and a further 24 females to Aroclor 1016 (17, 56). Furthermore, Arnold and colleagues performed a study on 80 female Rhesus monkeys exposed to different levels of Aroclor 1254 (8-11, 27, 62, 91). The results of these studies were used to assess the different exposure levels required to affect the reproductive system of the Rhesus monkey (to the left in Figure 5). It should be emphasized that these levels do not represent LOAELs (Lowest Observable Adverse Effect Level) or NOAELs (No Observable Adverse Effect Level), but are rather the results of three studies with a limited number of exposure groups. However, they give an idea of the difference in doses required to achieve the differ-

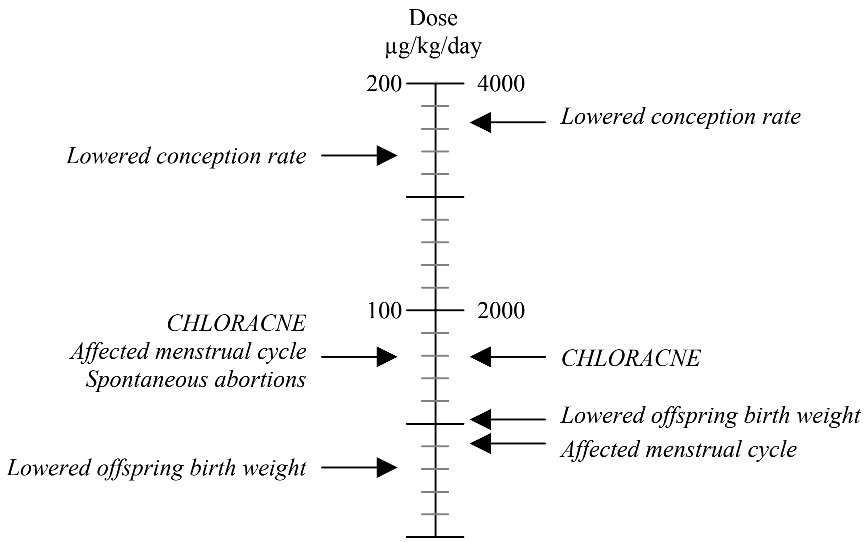


Figure 5. Effects of PCB exposure in Rhesus monkeys (left) and rodents (right).

ent reproductive outcomes.

LOAELs and NOAELs for reproductive outcomes in rodents after exposure to PCB have been put together by Golub *et al*. (42), and selected outcomes are shown in Figure 5. It can be seen that the doses required to affect the reproductive system of rodents is much higher than the doses required to result in effects among Rhesus monkeys. However, for both species, lowered birth weight and affected menstrual cycle (including menses duration and bleeding) seem to occur at lower exposure levels than that required to affect the conception rate.

TCDD has been shown to be foetotoxic for Rhesus monkeys when given orally at 1 µg/kg body weight in a single or divided dose between days 20 and 40 after conception (59), i.e. at much lower doses than required for reproductive effects of PCB. As with PCB exposure, the embryo/foetus of rodent species is generally not as sensitive to TCDD-induced prenatal mortality as the embryo/foetus of the Rhesus monkey (70).

MECHANISMS OF POC EFFECTS ON REPRODUCTIVE OUTCOMES

Most, if not all, TCDD effects are mediated through the Aryl hydrocarbon (Ah) receptor (35). Ah receptor agonists are capable of inducing a wide spectrum of biological effects at a number of different life stages. Indeed, TCDD has been found to affect reproductive tract development in ways both similar to and different from the actions of estrogens and anti-androgens.

Although coplanar PCBs interact with the Ah receptor in a way similar to TCDD, PCBs have also been shown to mimic or inhibit the production or action of a wide variety of steroids such as estrogens, progestins, androgens and adrenal steroids (31). However, though many PCBs are estrogenic, they typically have a weak binding affinity for the oestrogen receptor (ER) and relatively limited estrogenic potency, and only a few congeners have been shown to produce anti-estrogenic effects. PCBs can metabolise to hydroxy-PCBs, which can act through ER to induce estrogenic effects (81). The hydroxy-PCB levels in blood have been found to correspond to about a fifth of the PCB levels (83).

The thyroid hormones constitutes, together with the steroid hormones, the steroid-hormone receptor superfamily (92). It has been suggested that the thyroidal activity of PCB may be as important as, or even more important than, the estrogenic/anti-estrogenic effects when considering the potential impact on the reproductive system (35).

MEASURES OF FERTILITY

There are several biological processes involved in achieving a pregnancy in a specific menstrual cycle. In the female body, conception depends on the success of ovulation, fertilisation and implantation, whereas e.g. the sperm quality is one of several important factors in the male body. Furthermore, the conceptus needs to survive until the pregnancy is clinically recognisable. The term *fecundability* was first introduced by Potter and Parker (71) to denote the monthly chance of conception in the absence of contraception. It integrates reproductive effects of exposures to both parents and to the conceptus because it incorporates effects on all the biological processes required for procreation. Estimates of fecundability can be derived by assessing the time that elapses between the cessation of contraceptive use and clinical recognition of pregnancy, i.e. the *time to pregnancy* (93). Time to pregnancy can also be used as a dichotomous variable. This is often the case in a clinical context, where twelve months or more of unprotected intercourse without pregnancy is the usual definition of infertility.

The use of time to pregnancy has been found to be a useful tool for the assessment of reproductive effects of exposures in general (51), as well as for environmental exposures (15). Data can be collected retrospectively (51) by using self-administered questionnaires (97).

Once a pregnancy is clinically recognised, reproductive loss can be measured by the frequency of miscarriages and stillbirths.

REPRODUCTIVE EFFECTS OF POC IN HUMANS

Accidental high short-time exposure

Yusho and Yu-Cheng

In 1968, a mass intoxication of almost 2000 people on the Japanese island Kyushu occurred when rice oil was contaminated with Kanechlor that had leaked from a heating tube in a tank. The most predominant signs of intoxication were dermal changes, such as chloracne and pigmentation of the skin (49). The epidemic was named “Yusho”, which is Japanese for “oil disease”.

Figure 6. The Japanese island Kyushu.

In 1979, the Yusho-accident was repeated in Taiwan. The number of victims was again about 2000, and the epidemic was referred to as “Yu-Cheng” (Chinese for “oil disease”). The PCB/PCDF concentration in the Taiwanese oil was only about one tenth of that in the Japanese oil, but the Taiwanese patients consumed about 10 times as much oil as the Japanese patients, and the clinical manifestations seen in the Taiwanese patients were also very similar to those reported in Japan (49).

The number of studies investigating the reproductive function among women exposed in the Yusho and Yu-Cheng accidents is few to date. A higher rate of stillbirths has been found, but no evidence of an effect on miscarriages (96). Furthermore, there seem to be a mild interference with menstrual function, but no change in fertility. Infants born to affected Yu-Cheng mothers were found to be shorter and not lighter than control infants (72), and even though the women themselves did experience increased mortality, their children had a high perinatal mortality (48).

Seveso

In the summer of 1976, an accident occurred in a trichlorophenol reactor at a chemical plant in Seveso, Italy (82). An unexpected rise in temperature and pressure, together with the failure of a safety devise caused the contents of the reactor to vent directly into the atmosphere, creating a "toxic cloud" containing TCDD as well as other chemicals (19).

In 1996 a study on reproductive health in the Seveso women was launched (37). So far, the only results presented suggested that higher TCDD concentrations were associated with longer menstrual cycle among women who were premenarcheal but not among women who were postmenarcheal at the time of the accident (38). Other studies on the Seveso population have found that among infants born to this population there was an excess of females (63, 64), but no evidence of an effect was found on the duration of the gestational period or birth weight of the infants (82).

Long-time exposure through fish consumption

The Great Lakes Region

The ecosystems of the Great Lakes, on the American-Canadian border, have become contaminated with hazardous compounds such as PCBs and dioxins (32), and consumption of fatty fish from these waters serves as a major dietary source of exposure to PCBs (85). Several studies have been performed in order to investigate possible health effects of the consumption of fatty fish from the Great Lakes.

Figure 7. The Great Lakes, on the border of USA and Canada.

The Michigan Maternal Infant Cohort, or the Jacobson Cohort, was the first to assess the reproductive effects of eating contaminated fish from the Great Lakes. The cohorts were established in the early 1980's using a study population of pregnant women with moderate or no consumption of Lake Michigan fish, and their infants (39). It was found that PCB exposure, measured by fish consumption as well as cord serum PCB levels, predicted a shorter gestational period, lower birth weight and smaller head circumference. However, when using fish consumption during pregnancy as a measure of exposure, the investigators failed to replicate these results.

The New York State Angler Cohort consists of a group of Lake Ontario sport fishers (male as well as female) and their spouses (94). For both the women and the men in the cohort information was collected on years of fish consumption, frequency of fish consumption and estimated lifetime PCB exposure. Furthermore, for the women a lifetime estimate of fish consumption was calculated. No evidence of an effect of either male of female exposure was found on infecundity or foetal death, but increased menstrual cycle length as well as increased time to pregnancy was found for female exposure (Table 1).

Table 1. Results from the New York State Angler Cohort studies.

	Resolved infecundity	Unresolved infecundity	Delayed conception	Time to pregnancy	Foetal death	Menstrual cycle length
Maternal exposure						
Duration (years) of fish consumption	0(58)	0(58)		0(29) +(30)	0(61)	+(60)
Frequency of fish consumption in 1990-91	0(58)	0(58)		0(29) +(30)	0(61)	+(60)
Estimated lifetime PCB exposure				0(30)	0(61)	+(60)
Lifetime estimate of fish consumption (kg)					0(61)	
Paternal exposure						
Duration (years) of fish consumption			0(28)	0(30)		
Frequency of fish consumption in 1990-91			0(28)	0(30)		
Estimated lifetime PCB exposure			0(28)	0(30)		

0 = no evidence of an effect was found, "+" = an effect was found

In a study of 830 Michigan Anglers (male and female), Courval *et al.* (33) found an association between high lifetime sport-caught fish consumption and conception delay (ever having a time to pregnancy longer than 12 months) for male exposure only.

When studying 1115 women from Green Bay by Lake Michigan, Dar *et al.* (36) found that infant birth weight increased with fish consumption scores. These women were, however, not chosen due to presumed high PCB exposure, but were assumed to constitute a representative sample of all women in the area.

Swedish Fishermen's Family Cohorts

The major exposure source of POC in the Swedish population is the consumption of fatty fish caught in the Baltic Sea, off the east coast of Sweden (3, 12, 90). It has been shown that the estimated current dietary exposure to POC through fish consumption was comparable between groups of high consumers from the Baltic Sea and the Lake Ontario region (2). Swedish fishermen and their families have been found to eat more fish than the general Swedish population (46, 78, 79, 90). Therefore, fishermen's families from the east coast have been considered a suitable population when studying possible health effects of POC exposure. Since the fish caught in the waters off the west coast is considerably less contaminated (18), the west coast fishermen's families have been considered an appropriate reference group.

Cohorts of fishermen from both coasts have been established from different fishermen's organizations (46, 89). Cohorts of fishermen's wives and ex-wives have then been established by linkage of the fishermen cohorts to the national Swedish population register, and to registers at local parish offices (75). Linkage to the population register has also provided cohorts of sisters to the fishermen, excluding women who were already in the fishermen's wives cohorts (80). From the MBR, information on infants born to the fishermen's wives and sisters has been collected (78, 80). Furthermore, from a subset of the women in the east coast wives cohort, blood samples have been drawn and analysed for CB-153 (77). The available information has enabled studies on several reproductive, mainly post-birth, outcomes.

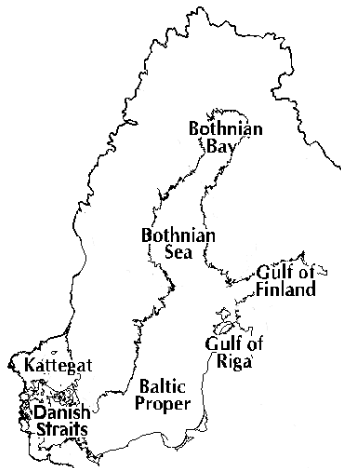


Figure 8. The Baltic Sea, off the east coast of Sweden, includes the Bothnian Bay, the Bothnian Sea, and the Baltic Proper.

In the cohort comparisons, infants to both fishermen's wives (78) and sisters (80) from the east coast were found to have lowered birth weight compared to women in the west coast cohorts. Within the east coast fishermen's wives cohort, probable childhood exposure (79), a high current Baltic Sea fatty fish consumption (79), as well as a high blood CB-153 concentration (77) also increased the risk of having a low birth weight infant. The infants in both east coast cohorts had smaller head circumference than those in the west coast cohorts (80). There was, however, no evidence of an effect on infant length (78, 80), and no increased risk for malformations (76). In a cohort comparison among the fishermen's wives, a decreased gender ratio (ratio of male to female infants) was found for the east coast cohort (78).

Exposure to the general population

The groups of fish consumers have been chosen as study populations since they are assumed to have a higher exposure to PCBs and dioxins than the general population. However, a relation between reproductive disorders and body burden of POCs have also been found in other groups of women: In a study of 207 Dutch women it was found that both cord and maternal plasma PCB levels were negatively associated with birth weight (69). Furthermore, among 89 women with repeated miscarriages, a positive association between POC concentrations in blood and number of miscarriages was found (40).

Aims of the thesis

- ❍ To investigate whether exposure to POC through the consumption of fatty fish from the Baltic Sea leads to a decreased fertility, measured as time to pregnancy.
- ❍ To investigate whether the same exposure implies an increased miscarriage risk.
- ❍ To assess the relation between blood levels of PCB and the reproductive outcomes time to pregnancy and miscarriages.

Materials and methods

STUDY COHORTS

To investigate possible effects of POC exposure on fertility, cohorts of east and west coast fishermen's wives and sisters were chosen. In Papers I-III and V, women born in 1945 or later were selected from previously established cohorts of east and west coast fishermen's wives (75). This selection by year of birth was done since younger women are more likely to have been pregnant at a later calendar date, and hence are more likely to remember the circumstances surrounding their pregnancies than are older women.

In order to establish the cohorts of fishermen's sisters, east and west coast fishermen cohorts (46, 89) were linked to the national Swedish population register by Statistics Sweden. As with the fishermen's wives, only women born in 1945 or later were included (Papers IV and V). Furthermore, women born after 1979 were excluded since they were considered to be too young to be sent a questionnaire on reproduction. Fishermen's sisters who were married to fishermen, and thus included in the fishermen's wives cohorts, were also excluded from the cohorts of fishermen's sisters (46 east coast and 302 west coast women).

DATA COLLECTION

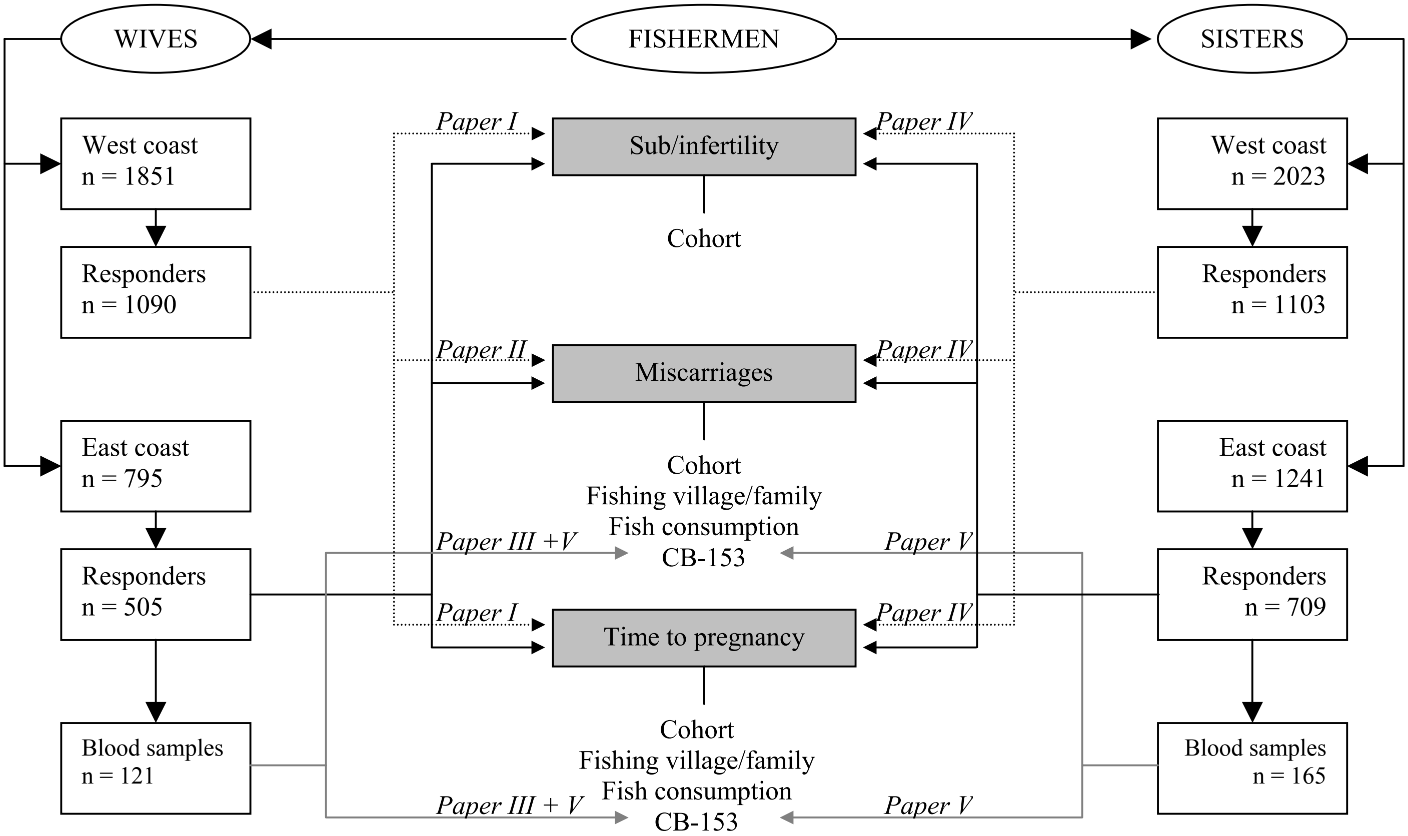
Questionnaires

The fishermen's wives

In 1997, a questionnaire was sent to the fishermen's wives. For each woman's five first pregnancies, information was collected on the use of contraceptives prior the pregnancy, time to pregnancy for those pregnancies that where planned, pregnancy outcome, and whether the pregnancy was a result of any medical treatment or a birth control failure. Furthermore, possible confounders such as working situation (working/not working, full/part time, shift work and night work) and heavy lifting was asked about for the woman, whereas other confounders such as smoking habits and coffee consumption was assessed for both the woman and her partner. The women were asked if they at any point in their life unsuccessfully had tried to conceive for a consecutive period of at least 12 months, and how many children they had. Furthermore, they were asked if they had grown up in either a fisherman's family or in a fishing village. Their current fish consumption was assessed as "never", "1-4 meals/month", "5-14 meals/month" and "at least 15 meals/month" for lunch and dinner separately. After sending two reminding letters to the non-responders, the response rate was still rather low (60%).

The fishermen's sisters

In 1999, a shorter questionnaire was sent to the fishermen's sisters. This time only the first planned pregnancy of each woman was inquired about. The questions asked in the sister questionnaire were similar to those in the wife questionnaire. No information was collected on the women's partners, but in addition to the informa-


WIVES
FISHERMEN
SISTERS
West coast
n = 1851
Responders
n = 1090
East coast
n = 795
Responders
n = 505
Blood samples
n = 121
West coast
n = 2023
Responders
n = 1103
East coast
n = 1241
Responders
n = 709
Blood samples
n = 165
Paper I
Paper IV
Sub/infertility
Cohort
Paper II
Paper IV
Miscarriages
Cohort
Fishing village/family
Fish consumption
CB-153
Paper III +V
Paper V
Paper I
Paper IV
Time to pregnancy
Cohort
Fishing village/family
Fish consumption
CB-153
Paper III + V
Paper V

tion collected from the fishermen's wives questionnaire, the fishermen's sisters were also asked about their alcohol consumption, and if they at any point in their life had experienced a miscarriage. Since the linkage to the population register was performed by Statistics Sweden, the woman's identity was not automatically available from the questionnaire. Hence, the women were asked if they were willing to participate in further studies, and if so to give consent to Statistics Sweden to reveal their identity. The women who gave such consent, but failed to supply a time to pregnancy, were sent a letter with the aim of collecting this information. After sending two reminders to the non-respondents, the response rate among the fishermen's sisters was similar to that among the fishermen's wives (56%).

In the sister questionnaire, both the east and west coast women were asked about their consumption of fatty Baltic Sea fish only. However, when analysing the data it became evident that information on the consumption of locally caught fatty fish was needed from the west coast women. An additional questionnaire on fish consumption was therefore sent to west coast fishermen's sisters whose identity was known. Out of 364 such women, 262 (72%) supplied information on their consumption of locally caught fatty fish.

Of the 709 east coast fishermen's sisters who replied to the first questionnaire, 203 women supplied a time to pregnancy as well as agreed to participate in further studies. These women were contacted and asked if they were willing to supply blood samples for analysis of CB-153 (see below). At the time of the blood sampling they were asked to fill out a second questionnaire regarding time to pregnancy and pregnancy outcome for all their pregnancies. Information was collected on the year of the pregnancy, use of contraceptives before conception, time to pregnancy, smoking habits, pregnancy outcome and, in case of live births, breast-feeding (months of breast-feeding only, breast-feeding together with additional formula, and breast-feeding combined with solids).

Blood sampling and analysis

For the east coast fishermen's wives cohort, blood had been drawn for 192 women in 1995, and the concentration of CB-153 in plasma had been determined (77). Of these 192 women, 121 had supplied a time to pregnancy.

Among the east coast fishermen's sisters, 203 women supplied a time to pregnancy and agreed to participate in further studies. These women were contacted and asked if they were willing to supply blood samples for analysis of CB-153. Twelve women were disinclined and 8 were unreachable. Blood was drawn from the remaining 183 women by two registered nurses. However, three women were excluded from the study since the nurses failed to draw enough blood for analyses of both CB-153 concentration and lipid content, and a fourth woman was excluded due to hepatitis infection. Thus, the concentration of CB-153 in serum was determined for 179 blood samples.

After the blood had been drawn from the east coast fishermen's sisters, a second questionnaire was administered by the nurses (see above). Although the women were chosen since they had supplied a time to pregnancy in the first questionnaire, there were now 14 women who claimed never to have had a planned pregnancy. This left 165 women, making for a total of 286 in a joint analysis of both fishermen's wives and sisters.

EXPOSURE

East coast cohort affiliation was considered as exposure, using the west coast cohorts as comparison cohorts. Originally, current Baltic Sea fatty fish consumption was intended to be used as a measure of exposure within the east coast fishermen's wives and sister cohorts. However, when analysing the material from the fishermen's sisters, indications of a protective effect was found for women with a high fish consumption. The effects of fatty fish consumption within the west coast cohorts were therefore also evaluated. For the east coast fishermen's wives, childhood exposure was defined as having grown up in a fishing village, whereas for the east coast fishermen's sisters, the definition also included women who had grown up in a fisherman's family.

The CB-153 concentrations analysed from the blood samples were used as measures of exposure. However, a woman's current concentration of CB-153 is not necessarily representative of her concentration at the time when she tried to conceive. A model for estimating past concentrations was available (77) (see also *Statistics*), and the estimated past CB-153 concentrations were therefore used as exposure within the east coast cohorts.

OUTCOMES

The pregnancy of interest was the first planned pregnancy of the fishermen's wives and sisters. For the fishermen's wives, this was chosen from the five first pregnancies, whereas for the fishermen's sisters, this was the only pregnancy for which information was available. The outcome time to pregnancy was determined for the fishermen's wives by asking "How many months of trying did it take you to become pregnant?". For the fishermen's sisters time to pregnancy was assessed by a line of questions: "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?"

Both fishermen's wives and sisters were asked about the outcome of the pregnancy, and pregnancies ending in miscarriages were identified. Furthermore, both wives and sisters were asked if they had ever unsuccessfully tried to conceive for a consecutive period of at least 12 months. The women who answered yes to this question were considered subfertile. Some of the fishermen's wives had answered no to this question, or had not answered at all, but had given a time to pregnancy exceeding 12 months for at least one of their five first pregnancies. These women were also considered subfertile. Among the fishermen's wives, subfertile women who had no children were considered infertile. This outcome was, however, not used for the fishermen's sisters, since most of these women were still of reproductive age.

NON-RESPONDENTS

The response rate to both questionnaires was very low, and information from other sources was used to investigate whether selection bias was introduced. For the fishermen's wives information was available from a previous study which included data from MBR (78). However, the personal identification number that is unique for every Swedish citizen was not included in this data, and the non-respondent analysis could therefore be performed only on the group of women whose birth-date was unique. Furthermore, women who were born before 1955 were excluded from the non-respondent analyses since it was considered likely that they would have given birth before 1973, which was the starting year of MBR. Thus, the non-respondent analyses included 612 respondents and 297 non-respondents.

The analyses among the fishermen's wives revealed that the age distribution was similar among respondents and non-respondents (Paper I). Furthermore, the percentage of women who had been pregnant at least once, or had ever given birth to a low birth weight child (<2500 g) or a child with malformation(s), differed only marginally between the respondents and non-respondents to this questionnaire, as did smoking habits and the percentage of women gainfully employed.

The cohorts of fishermen's sisters were established by Statistics Sweden, and the identities of the women included in the cohorts were known only to Statistics Sweden (with the exception of those respondents who agreed to let their identity be known). Therefore, a non-respondent analysis could not be performed on an individual level. However, information on a group level was available from different registers of Statistics Sweden. It was found that the non-respondents tended to be older and less educated than the respondents (Paper IV). Furthermore, there was a higher percentage of women who had never given birth among the non-respondents compared to the respondents.

Of 1103 west coast fishermen's sisters who answered the questionnaire on time to pregnancy, 364 agreed to participate in further studies, and were sent the questionnaire on consumption on fish from the west coast of Sweden (Paper IV). There was a higher percentage of women who had experienced a miscarriage among those who agreed to participate in further studies than among those who did not. With respect to other reproductive outcomes, no differences were found between the two groups of women.

Among 709 east coast fishermen's sisters who answered the questionnaire on time to pregnancy, only 183 women participated in the study regarding CB-153 concentrations and fertility (Paper V). The women were excluded if they had not supplied a time to pregnancy, had not agreed to participate in further studies, or if they were disinclined to supply a blood sample. Information from the time to pregnancy questionnaire was used to compare the participants and non-participants. It was found that they did not differ with respect to time to pregnancy, current age, or current consumption of Baltic Sea fatty fish. There were, however, some differences in miscarriage and subfertility rates. Furthermore, the participants were more likely to have grown up in a fisherman's family or a fishing village.

STATISTICS

Estimation of past CB-153

Developing a model

Exposure to CB-153 leads to accumulation of the chemical in the body. The total amount of CB-153 in the body at a specific time is called the *body burden* of CB-153. For highly lipid soluble compounds with long biological half-lives, such as CB-153, there is an equilibrium between the concentration in blood lipids and the concentration in adipose tissue, in which the main body burden of CB-153 is distributed. If the exposure is constant over time, the body will over time reach a *steady state* between uptake and metabolism/excretion, i.e. the concentrations in adipose tissue and blood lipids remains the same (cf Figure 9).

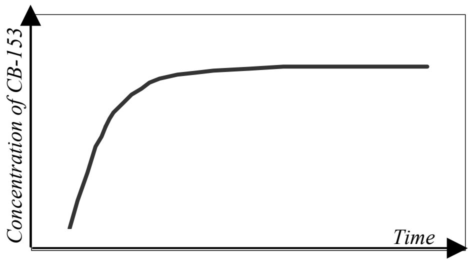


Figure 9. With constant exposure, the body will reach a steady state between uptake and metabolism/excretion.

At each chosen point in time (t), the plasma concentration of CB-153 (CB) can be calculated using the formula

$$CB(t) = A\left(1 - e^{-Bt}\right) \qquad \text{(i)}$$

where A is the plasma lipid concentration at the steady state and B = ln(2)/$T_{½}$,, where $T_{½}$ is the half-life of CB-153.

Since CB-153 is lipid soluble, it is excreted through breast milk in lactating women, leading to a reduction in the body burden during breast-feeding (55, 73, 74, 84). After breast-feeding the woman will have the same lipid concentration of CB-153 in plasma as she had *x* years previously. Furthermore, after the cessation of breast-feeding her body burden will increase in the same manner as it did before the lactational period, and eventually reach steady state again. Therefore, one way to look at this reduction is that the woman "falls backwards" on her body burden curve.

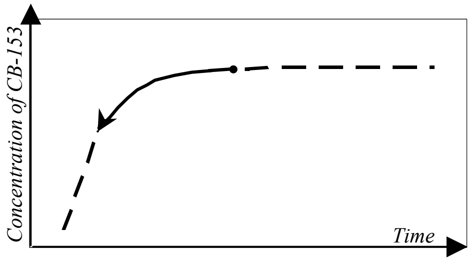


Figure 10. Reduction of body burden of CB-153 due to lactation.

Assuming that the reduction in body burden due to breast-feeding is q, the concentrations before and after lactation are related as follows

$$q \cdot CB(t_{lact}) = CB(t_{lact} - x) \qquad \text{(ii)}$$

where t_{lact} is the time point when lactation starts. By combining (i) and (ii) the resulting "decrease in age" can be calculated by:

$$x = t_{lact} + \frac{1}{B}\ln\left(1 - q\left(1 - e^{-Bt_{lact}}\right)\right) \qquad \text{(iii)}$$

By using this formula for each time the woman lactated, the age at which the woman first had the plasma CB-153 concentration that she had when the blood was drawn (t_{blood}) can be calculated. Thus,

$$CB(t_{blood}) = CB\left(t_{blood} - \sum_i x_i\right) \qquad \text{(iv)}$$

Combining this with (i) gives

$$CB(t_{blood}) = A\left(1 - e^{-B\left(t_{blood} - \sum_i x_i\right)}\right) \qquad \text{(v)}$$

and the woman's steady state is given by

$$A = \frac{CB(t_{blood})}{1 - e^{-B\left(t_{blood} - \sum_i x_i\right)}} \qquad \text{(vi)}$$

Equations (i) and (vi) thus gives the estimated concentration at time t_{est} to be

$$CB(t_{est}) = \frac{CB(t_{blood})}{1 - e^{-B\left(t_{blood} - \sum_i x_i\right)}}\left(1 - e^{-Bt_{est}}\right) \tag{vii}$$

For the women in the fishermen's family cohorts, as well as for the general Swedish population, the main exposure to CB-153 is through the consumption of POC-contaminated fatty fish from the Baltic Sea (3, 12, 90). Since the concentrations of POC in the environment have been decreasing over the past decades (20), constant fish consumption does not imply a constant exposure. Thus (vii) must be adjusted for the environmental decrease. Assuming a yearly decrease of r between the year of interest and the year of blood sampling, the plasma concentration of CB-153 can be estimated using the following formula.

$$CB(t_{est}) = \frac{CB(t_{blood})}{1 - e^{-B\left(t_{blood} - \Sigma x_i\right)}}\left(1 - e^{-Bt_{est}}\right)\left((1 + r)^{t_{blood} - t_{est}}\right) \tag{viii}$$

Validating the model

The model for estimating past CB-153 concentrations described above assumes a stable background exposure as well as constant individual intake of Baltic Sea fatty fish. Furthermore, the model depends on three parameters: The environmental reduction of CB-153 concentration, the reduction of a woman's body burden of CB-153 due to lactation, and the biological half-life of CBN-153. In Paper III, the model was validated using serum samples from a rubella screening program during the time period 1975-1991 ("actual past concentrations"), which were available for 20 of the 121 fishermen's wives in the study. Based on the literature, but also on the results of the validation, the model parameters were set as follows. 1) The environmental reduction of CB-153 concentration, and specifically the reduction of CB-153 concentration in Baltic Sea fatty fish, was assumed to be 3% yearly from 1976 and onwards. Before 1976 it was assumed that there was no reduction. 2) For each lactational period lasting less than six months, it was assumed that the woman's body burden was decreased by 20%, whereas lactational periods exceeding six months decreased the body burden by 30%. 3) The biological half-life of CB-153 was assumed to be 5 years.

In Paper V, serum samples for validation were obtained for 13 fishermen's sisters. One of the 20 fishermen's wives previously used for validation was excluded since the serum sample was not obtained during a pregnancy, leaving a total of 32 observations for a second validation of the model. The median time elapsed between the two sampling times was 9 (range 4-20) years (8 [4-20] years for the fishermen's wives and 10 [7-14] years for the fishermen's sisters).

Each woman's current concentration of CB-153 (from 1995 for the fishermen's wives and from 2000 for the fishermen's sisters) was used to estimate the concentration at the time when the actual past concentration was determined. The correlations between actual and estimated past CB-153 concentrations were similar to those between actual past and current CB-153 concentrations (Table 2; Figure 11). However, when trichotomizing all three CB-153 concentrations at the 33rd (0.88 ng/ml plasma) and 67th (1.49 ng/ml plasma) percentile of the actual past CB-153 concentrations, κ (the kappa statistic) (7) between estimated past and actual past concentrations was higher than that between the current and actual past concentrations. Furthermore, the mean pairwise difference was smaller for the estimated past concentrations than for the current, although the standard deviations were similar. Thus, even though the rank correlation between the current and estimated past concentrations was high (r_S=0.96), the estimated past concentrations seemed to agree better with the actual past than did the current concentrations.

Table 2. Validation of a model for estimating past CB-153 concentrations.

		Agreement with actual past concentrations			
	Median CB-153 (ng/ml plasma)	Spearman's correlation	κ	Mean difference[a]	Standard deviation[a]
Wives					
Estimated past	1.26	0.79	0.53	0.23	0.63
Current	0.71	0.72	0.26	-0.33	0.68
Sisters					
Estimated past	1.22	0.69	0.26	-0.13	0.51
Current	0.64	0.69	0.15	-0.57	0.41
Total					
Estimated past	1.25	0.71	0.44[b]	0.08[b]	0.60[b]
Current	0.70	0.64	0.20	-0.43	0.59

[a] *Statistics based on individual differences on continuous data.*
[b] *Results presented in Paper V.*

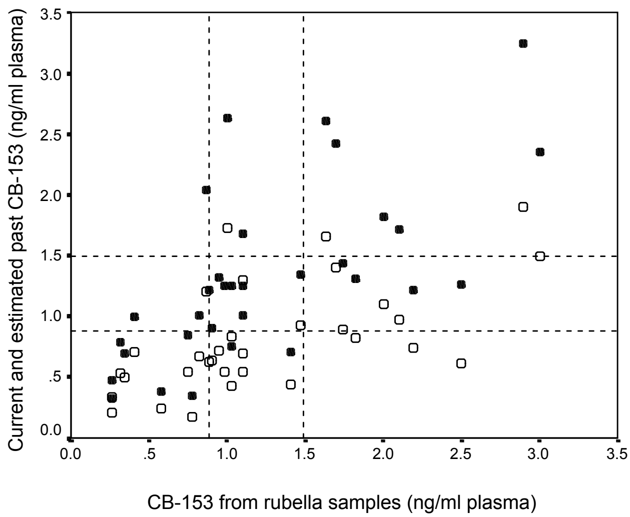


Figure 11. Current (open circles) and estimated past (filled circles) vs. actual past CB-153 concentrations. Reference lines at the 33rd and 67th percentile of the actual past CB-153 concentrations.

Analysis of time to pregnancy

Since time to pregnancy describes the time to an event (a wanted pregnancy), Cox regression have been suggested as a suitable method for comparing populations with different exposures (15, 52). In the analyses of time to pregnancy in the fishermen's wives study (Papers I and III), the procedure PHREG with discrete handling of ties in the statistical software SAS (version 6.12) was used to calculate the effect estimate, Success Rate Ratio (SuRR). The discrete handling of ties implies that when two or more events happen at the same time (ties) there is no underlying ordering (6) which is true for time to pregnancy data since a woman can only conceive once in each menstrual cycle. The Cox regression model then translates into the Cox's model for discrete-time data, which likelihood equation is similar to that for the proportional odds model. This implies that the model can also be applied using logistic regression to a database of cycles and estimating the per cycle relative odds of pregnancy (15). Thus, since the number of ties in the fishermen's sisters data was to large for SAS to handle (specifically, many women got pregnant after only one month of trying), logistic regression was used to calculate the Success Odds Ratio (SOR) for time to pregnancy (Papers IV and V). It should be noted that both the SuRR and the SOR measures a positive event. Thus, a value > 1 indicates a prolonged time to pregnancy for the *reference* group.

For illustration, the results produced by the logistic regression approach to the results from the Cox regression in the specific setting of time to pregnancy among fishermen's wives and sisters were compared. One hundred times to pregnancy were randomised according to a distribution that was set from the actual times to pregnancy of the fishermen's wives and sisters (see Appendix). Using different distribution shift factors, five sets of 100 times to pregnancy were then randomised to enable calculations of SORs and SuRRs. As expected from the likelihood theory mentioned above, the results from the two different methods of analysis are very similar (Table 3). Indeed, the notation "SuRR" may be misleading since the effect estimate calculated is in fact an odds ratio (OR). To better enable comparison between the fishermen's wives and sisters, the effect estimates for the fishermen's wives are presented as both SuRR and SOR in the Results section of this thesis.

Table 3. Effect estimates of survival calculated using logistic regression and Cox regression.

Distribution shift factor	Median time to pregnancy (months)	Logistic regression SOR (95% CI)	Cox regression SuRR (95% CI)
0.50	1	0.09 (0.05-0.17)	0.09 (0.05-0.17)
0.75	1	0.42 (0.28-0.62)	0.42 (0.28-0.62)
1.00	2	1.00	1.00
1.25	4	2.02 (1.41-2.88)	2.00 (1.40-2.86)
1.50	6	2.75 (1.89-4.01)	2.73 (1.88-3.96)
2.00	13	4.49 (3.01-6.71)	4.43 (2.97-6.60)

From the effect estimates a *change in time to pregnancy* can been calculated in such a way that e.g. SOR 0.90 implies that there is an 11% (0.90=1/1.11) increase in time to pregnancy for the exposed group compared to the unexposed group, whereas

SOR 1.10 implies an 10% decrease in time to pregnancy for the exposed group compared to the unexposed group.

Each woman was allowed to contribute with only one pregnancy. This was to eliminate interference from a correlation with the time to pregnancy of succeeding pregnancies, but also to avoid under-representation of couples who required a long time to achieve pregnancy. Some of the fishermen's sisters had ticked the "no" boxes for achieving pregnancy in the first or second month, but had not given a time to pregnancy. These women were included in the time to pregnancy-analyses but were censored at two months. Since the question assessing time to pregnancy was different for the fishermen's wives, no such censoring was necessary for these women.

Among the fishermen's sisters, all women who stated that their pregnancy was a result of medical treatment were included in the analyses (Papers IV and V). However, regardless of their time to pregnancy given in the questionnaire, it was assumed that the actual time to pregnancy was longer than 12 months, since this is normally the earliest time point when medical treatment is introduced. This assumption was not originally made for the fishermen's wives (Papers I and III), but in the calculation of the SOR presented in the Results section of this thesis, 51 fishermen's wives (east, n=12; west, n=39) were assumed to have had a time to pregnancy longer than 12 months (i.e. censored time to pregnancy) even though they stated something else in the questionnaire.

Analysis of subfertility and miscarriages

For comparisons of subfertility, infertility, and miscarriage rates logistic regression was used to calculate OR and 95% confidence intervals (CI). All women who at one point in their life unsuccessfully had tried to conceive for a consecutive period of at least 12 months were asked if they had consulted a physician as a result of this. If an investigation was made, and if this revealed decreased female and/or male fertility, the couple was considered "medically sterile". In the subfertility analysis for the fishermen's wives (Paper I) the medically sterile couples were excluded, but in the same analysis for the fishermen's sisters (Paper IV) they were not. For comparability, in the Results section of this thesis results are presented where the sterile couples are included as well as excluded.

For both fishermen's wives and sisters, miscarriage was determined by using information on the outcome of the first planned pregnancy (miscarriages and stillbirths). However, among the fishermen's sisters the miscarriage rate was compared to the number of live births (Paper IV), whereas for the fishermen's wives the comparison was made to the number of live births, extrauterine pregnancies, and induced abortions (Paper II). Still ongoing pregnancies were, however, excluded in all miscarriage analyses. For the fishermen's wives, the miscarriage analyses were stratified on the time of the miscarriage: early miscarriages (before week 12), late miscarriages (between weeks 12 and 28) and stillbirths (after week 28). Only the total risk was calculated for the fishermen's sisters. In the Results section of this thesis, total miscarriage risk for both the fishermen's wives and sisters have been calculated using live births, extrauterine pregnancies and induced abortions as well as live births only as comparisons. Furthermore, miscarriage risks stratified by gestational week have been calculated.

Concentrations of CB-153 for women with live births and miscarriages are described using box-plots. The box represents the interquartile range (lower border represents the 25^{th} percentile and upper border the 75^{th} percentile), and the line across the box represents the median. The highest and lowest values, excluding outliers, are represented by lines that extend from the box. Outliers are defined as observations that are further from the lower or upper border of the box than 1.5 times the interquartile range, and are represented by open circles. The concentrations of CB-153 for women with live births and miscarriages are compared using the Mann-Whitney U-test, which is non-parametric.

Assessing confounders

In the questionnaires, information on several possible confounders (see above) was collected. These confounders were included in multivariate models if inclusion changed the effect estimate by more than 10%. However, in Paper I some of the possible confounders were never tried in multivariate models since they did not show any univariate effect. In Papers II-V the possible confounders were not tested for univariate effects.

Results

TIME TO PREGNANCY

Cohort comparisons

For the fishermen's wives a somewhat prolonged time to pregnancy among the east coast women compared to the west coast women was found (median 3 vs. 2 months; Paper I; Table 4). A stratified analysis revealed that this was due to an increased time to pregnancy among east coast women who smoked more than 10 cigarettes per day (heavily smoking). These women had a median time to pregnancy of 4 months, compared to 2 months among the heavily smoking women on the west coast. Among non/light smoking women (0-9 cigarettes per day) the median time to pregnancy was 2 months for both the east and west coast women. For the fishermen's sisters no effect of cohort affiliation was found (median time to pregnancy 2 months in both the east and west cohort; Paper IV; Table 4).

Table 4. Cohort comparisons (east vs. west coast) of time to pregnancy among 1335 fishermen's wives and 1427 fishermen's sisters.

	SuRR (95% CI)	SOR (95% CI)
Wives	0.86 (0.75-0.99)[a]	0.92 (0.80-1.05)
0-9 cigarettes/day	0.95 (0.81-1.12)[a]	1.04 (0.89-1.22)
≥ 10 cigarettes/day	0.68 (0.51-0.91)[a]	0.65 (0.48-0.89)
Sisters	---[b]	0.99 (0.87-1.14)[c]
0-9 cigarettes/day	---[b]	0.88 (0.74-1.05)
≥ 10 cigarettes/day	---[b]	1.24 (0.98-1.56)

[a] *Results presented in Paper I* [b] *Not calculated due to a too large number of ties*
[c] *Results presented in Paper IV*

As stated in *Statistics*, the SORs presented were calculated assuming that all pregnancies that were the result of medical treatment had a time to pregnancy longer than 12 months. This assumption was originally not made for the fishermen's wives, which is why the SuRRs and SORs in Table 4 differ somewhat from each other.

Table 5. Effect of smoking (10 or more cigarettes/day vs. 0-9 cigarettes/day).

	East coast SOR (95% CI)	West coast SOR (95% CI)
Wives	0.64 (0.49-0.84)	1.01 (0.83-1.25)
Sisters	1.11 (0.89-1.38)	0.80 (0.65-0.95)

If heavily smoking east coast fishermen's wives had higher CB-153 concentrations than did non- and light smoking east coast fishermen's wives, the results from the stratified analyses could be explained by higher exposure in the heavily smoking women. However, in the subgroup of women who supplied blood samples for analyses of CB-153, the concentrations of CB-153 were lower among the heavily smoking fishermen's wives than among the non/light smoking fishermen's wives (Figure 12). Within the east coast fishermen's wives cohort there was an effect of smoking habits on time to pregnancy that was not present in the west coast cohort (Table 5). The opposite was true for the fishermen's sisters, where an effect of smoking was found for the west coast but not for the east coast women.

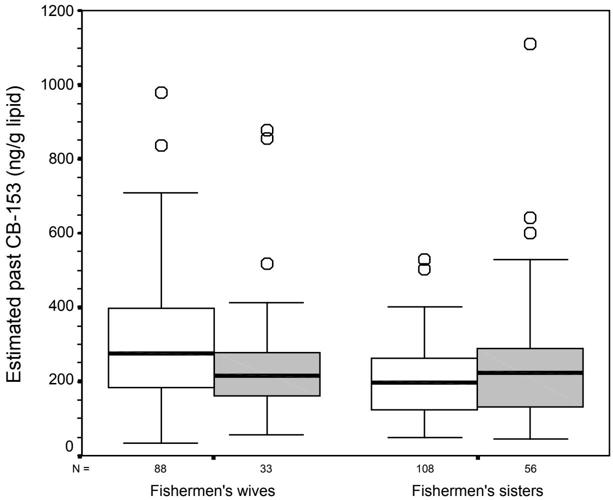


Figure 12. CB-153 concentrations among non/light (white boxes) and heavily smoking women (grey boxes).

Women who never conceived were originally not included in the time to pregnancy-analyses since no information on confounders was available for the time when they tried to conceive. However, all women were asked if they at any point in their life unsuccessfully had tried to conceive for a consecutive period of 12 months or more. When fishermen's wives (east coast, n=40; west coast, n=37) and sisters (east coast, n=35; west coast, n=41) who answered yes to this question but never supplied a time to pregnancy were included in a time to pregnancy-analysis by letting their time to pregnancy be greater than 12 months (i.e. censored at 12 months), the effect of east coast cohort affiliation was even more apparent among the fishermen's wives (SOR 0.80 [95% CI 0.70-0.92] for the whole cohort, and SOR 0.57 [0.43-0.77] for the heavily smoking women). For the fishermen's sisters the effect estimate changed only slightly (SOR 0.96 [0.84-1.09]).

All time to pregnancy-analyses were performed using the time to pregnancy of each woman's first *planned* pregnancy, regardless of the parity of that pregnancy. When restricting the analyses to pregnancies of parity one, the result was similar for the fisherman's wives (SOR 0.92 [95% CI 0.80-1.07]) but changed slightly for the fisherman's sisters (SOR 1.06 [0.90-1.24]). When using parity as a confounder, the effect estimates remain similar to the unadjusted ones (SOR 0.92 [0.80-1.05] for the fishermen's wives and SOR 1.00 [0.87-1.15] for the fishermen's sisters).

Each woman was allowed to contribute with only one pregnancy. However, for the fishermen's wives, information was available on each woman's five first pregnancies. When using all times to pregnancy as individual observations, regardless of the number of infants born to each woman, the effect estimate was only slightly changed, although the confidence interval was narrower due to the increased amount of observations (SOR 0.90 [95% CI 0.83-0.98]).

Although the fishermen's wives had all been married to a fisherman, not all of them achieved their first planned pregnancy with a fisherman. However, when stratifying on the profession (fisherman/not fisherman) of the partner, similar effect estimates were obtained for women whose partners were fishermen (SOR 0.91 [95% CI 0.77-1.07]) and those whose partners were not (SOR 0.90 [0.69-1.18]). Furthermore, adjusting the crude SOR for the profession of the partner only slightly changed the effect estimate (SOR 0.91 [0.79-1.04]).

The question assessing time to pregnancy differed in the wife and sister questionnaires, allowing the fishermen's wives, but not the sisters, to give a time to pregnancy of zero months. Whether women who stated that their time to pregnancy was zero months in reality took as long time to conceive as those who stated that their time to pregnancy was one month can only be speculated on. Letting zero months times to pregnancy be included in the analysis as one month times to pregnancy changed the effect estimate only slightly (SOR 0.87 [95% CI 0.75-1.01]), as did exclusion of these observations (SOR 0.88 [0.75-1.03]).

Women who conceive while using birth control may change their feelings about the pregnancy and report it as planned even though it is not, resulting in potential birth control failure bias (86). These women tend to report their time to pregnancy as one month (or zero months for the fishermen's wives). However, excluding these observations did not change the effect estimates more than marginally (SOR 0.96 [95% CI 0.80-1.15] for the fishermen's wives and SOR 0.98 [0.82-1.17] for the fishermen's sisters).

Childhood exposure

Growing up in a fisherman's family and/or a fishing village (Figure 13) was considered as a proxy for childhood exposure. Among the fishermen's wives, east coast women with this exposure had a decreased time to pregnancy compared to those east coast women who had not (median time to pregnancy 2 months for all exposed groups vs. 3 months for all unexposed groups; Table 6). This was not seen among the fishermen's sisters, where the median time to pregnancy for both exposed and unexposed groups was 2 months.

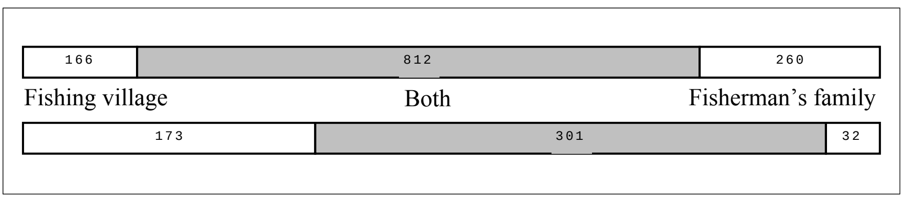


Figure 13. Childhood exposure among fishermen's sisters (top) and wives (bottom).

Table 6. Proxies for childhood exposure, and time to pregnancy among 399 east coast fishermen's wives and 562 east coast fishermen's sisters.

	Wives		Sisters
Grown up in a…	SuRR (95% CI)	SOR (95% CI)	SOR (95% CI)
fishing village	1.32 (0.94-1.86)[a]	1.34 (0.95-1.90)	1.19 (0.95-1.49)
fisherman's family	1.16 (0.80-1.68)	1.23 (0.85-1.79)	1.06 (0.85-1.31)
either	1.24 (0.91-1.68)	1.24 (0.91-1.70)	1.12 (0.89-1.40)[b]

[a] *Results presented in Paper I* [b] *Results presented in Paper IV*

Fish consumption

For the east coast fishermen's wives, no effect on time to pregnancy was found for those women who had a high (≥2 meals/month) current consumption of fatty Baltic Sea fish compared to those who did not eat any fatty Baltic Sea fish (SuRR 1.07 [95% CI 0.90-1.27]; Paper I). Among the east coast fishermen's sisters, there was a tendency towards a protective effect for women with medium (1-1.5 meals/month; SOR 1.16 [0.88-1.53]; Paper IV) and high (≥2 meals/month; SOR 1.27 [0.96-1.69]) compared to low fish consumption (≤0.5 meals/month). However, no significant dose-response trend was found.

In order to enable comparisons between the fishermen's wives and sisters, SORs for high (≥2 meals/month) compared to no fatty fish consumption are presented in Table 7. Since the results point towards a protective, rather than hazardous, effect of consumption of fatty fish among the east coast fishermen's sisters, analyses on the west coast women were also performed. The same indications of a protective effect are found for the west coast fishermen's sisters also. However, no effect is found for the fishermen's wives, regardless of cohort affiliation.

Table 7. Time to pregnancy and high (≥2 meals/month) vs. no (0 meals/month) consumption of fatty fish from the Baltic Sea.

	Median time to pregnancy		
	Low consumption	High consumption	SOR (95% CI)
Wives			
East coast	2 months	2 months	1.02 (0.72-1.44)
West coast	2 months	2.5 months	0.79 (0.56-1.09)
Sisters			
East coast	2 months	1 months	1.30 (0.97-1.74)
West coast	2 months	1 months	1.32 (0.98-1.77)

CB-153

Due to a programming error in the estimation of past CB-153, the ranges for low, medium and high exposure, as well as the SuRR, presented in Paper III are incorrect. However, the risk estimates based on the correct estimated past CB-153 are consistent with the results presented in Paper III, and show no effect of exposure for the medium (SuRR 0.81 [95% CI 0.49-1.35]) or high (SuRR 0.97 [0.75-1.24]) compared to the low exposure group. These results, as well as the SOR calculated for the east coast fishermen's wives, differ from those found among the east coast fishermen's sisters, where there seemed to be a protective, rather than hazardous, effect of high concentrations of estimated past CB-153 concentration (Table 8).

Table 8. CB-153 and time to pregnancy among 121 fishermen's wives and 165 fishermen's sisters.

		Estimated past CB-153 (ng/g lipid)	Crude SOR (95% CI)	Adjusted[a] SOR (95% CI)
Wives	Low	34-194	1.00	1.00
	Medium	195-316	0.80 (0.47-1.36)	0.95 (0.54-1.66)
	High	326-980	0.91 (0.53-1.58)	0.94 (0.54-1.62)
Sisters	Low	45-148	1.00	1.00
	Medium	149-239	1.51 (0.93-2.45)[b]	1.62 (0.98-2.66)[b]
	High	243-1111	1.54 (0.95-2.49)[b]	1.82 (1.10-3.01)[b]
Total	Low	24-178	1.00	1.00
	Medium	181-267	1.27 (0.89-1.82)[b]	1.41 (0.98-2.03)[b]
	High	271-1111	1.42 (0.99-2.03)[b]	1.59 (1.10-2.30)[b]

[a] *Adjusted for smoking/non-smoking* [b] *Results presented in Paper V*

SUBFERTILITY AND INFERTILITY

There was no significant effect of cohort affiliation on subfertility, neither when analysing the complete material, nor when excluding couples that were diagnosed sterile by a physician (Table 9). Smoking was found to be an effect modifier among the fishermen's wives, where an increased risk for subfertility was found among heavily smoking east coast women (Table 10). This was, however, not seen among the fishermen's sisters.

Table 9. Subfertility in 1230 fishermen's wives and 1797 fishermen's sisters.

	Sterile couples excluded			Sterile couples included		
	n	%	OR (95% CI)	n	%	OR (95% CI)
Wives						
West coast	269	36	1.00	327	39	1.00
East coast	133	39	1.15 (0.88-1.50)[a]	173	44	1.25 (0.98-1.60)
Sisters						
West coast	134	13	1.00	207	19	1.00
East coast	83	13	0.99 (0.73-1.31)	141	20	1.08 (0.85-1.37)[b]

[a] *Results presented in Paper I* [b] *Results presented in Paper IV*

For both fishermen's wives and sisters, subfertility was assessed by the question "have you at some point in your life unsuccessfully tried to conceive for 12 months or more". However, for the fishermen's wives, the women who had given a time to pregnancy exceeding 12 months for at least one of their five first pregnancies were also defined as subfertile, even if they had answered no to the question on subfertility (east, n=34; west, n=67), or if they had not answered at all (east, n=25; west, n=18). When excluding these 126 women, the subfertility risk was increased (OR 1.34 [95% CI 1.03-1.74]; sterile couples included).

Table 10. Subfertility (excluding medically sterile couples) among 1084 fishermen's wives and 1666 fishermen's sisters.

	Non or light smokers			Heavy smokers		
	n	%	OR (95% CI)	n	%	OR (95% CI)
Wives						
West coast	226	36	1.00	39	34	1.00
East coast	82	33	0.89 (0.65-1.21)[a]	49	54	2.36 (1.34-4.16)[a]
Sisters						
West coast	75	13	1.00	47	18	1.00
East coast	40	12	0.97 (0.64-1.46)	34	16	0.85 (0.52-1.37)

[a] *Results presented in Paper I*

For the fishermen's sisters, the median year of birth (1957) was used to stratify the material. Among the older women there was an indication of an increased subfertility risk (OR 1.18 [95% CI 0.85-1.65]; Paper IV) that was not found among the younger women (OR 0.98 [0.69-1.39]). The same effect was found among the fishermen's wives when stratifying at the median year of birth (OR 1.43 [0.99-2.08] and OR 0.91 [0.62-1.33] for women born before and after 1953, respectively; previously unpublished data).

When excluding couples that had been diagnosed sterile by a physician, an increased infertility risk was found for the east coast fishermen's wives compared to the west coast ones (OR 2.49 [95% CI 1.05-5.92]; Paper I). Infertility was not investigated among the fishermen's sisters, since most of these women were still of reproductive age.

MISCARRIAGES

Cohort comparisons

East coast cohort affiliation seemed to decrease, rather than increase, the miscarriage rate compared to the live birth rate (Table 11), as well as the rate of live births, extrauterine pregnancies, and induced abortions (Table 12). Among the fishermen's wives the decrease in risk seemed to occur for the early miscarriages (before gestational week 12), whereas the lowered risk occurred for the late miscarriages (between weeks 12 and 28) for the fishermen's sisters.

Table 11. Cohort comparisons (east vs. west coast) of miscarriages and stillbirths vs. live births.

	East coast n (%)	West coast n (%)	OR (95% CI)
Wives	28 (7)	89 (9)	0.68 (0.44-1.06)
Sisters	36 (7)	66 (8)	0.86 (0.57-1.31)[a]
Total	64 (7)	155 (9)	0.76 (0.56-1.03)

[a] *Results presented in Paper IV*

The fishermen's sisters were asked not only about the outcome of their first planned pregnancy, but also if they had ever experienced a miscarriage or a stillbirth. The result from this analysis was similar to those achieved from the analyses of first planned pregnancy outcome, with a tendency towards a decrease in risk for the east coast women (OR 0.83 [95% CI 0.67-1.04]).

Table 12. Cohort comparisons (east vs. west coast) of miscarriages and stillbirths vs. live births, extrauterine pregnancies and induced abortions among fishermen's wives and sisters.

	Before week 12	Weeks 12-28	After week 28	Total
Wives	0.48 (0.26-0.92)[a]	0.89 (0.44-1.80)[a]	1.36 (0.44-4.19)[a]	0.69 (0.44-1.07)
Sisters	1.03 (0.51-2.09)	0.71 (0.37-1.35)	---[b]	0.77 (0.48-1.23)
Total	0.63 (0.39-0.99)	0.79 (0.49-1.27)	0.76 (0.27-2.17)	0.70 (0.51-0.97)

[a] *Results presented in Paper II* [b] *None of the east coast sister experienced a stillbirth*

Childhood exposure

In the east coast cohorts, a slight protective effect on the miscarriage rate (compared to the live birth rate) of growing up in a fishing village and/or fisherman's family was found among the wives (OR 0.53 [95% CI 0.15-1.79]) as well as the sisters (OR 0.89 [0.44-1.81]; Paper IV).

Fish consumption

Among the east coast fishermen's wives, no increase in miscarriage risk was found for women who ate at least 2 meals of fatty Baltic Sea fish per month compared to those who did not eat fatty Baltic Sea fish (OR 0.62 [95% CI 0.23-1.66]; Paper II). Similarly, among the east coast fishermen's sisters no increased risk was found for medium (1-1.5 meals/month; OR 0.88 [0.37-2.09]; Paper IV) or high (≥2 meals/month; OR 0.73 [0.29-1.80]) current fatty Baltic Sea fish consumption compared to low (≤0.5 meals/month).

In order to enable comparisons between the fishermen's wives and sisters, SORs for high (≥2 meals/month) compared to no fatty fish consumption are presented in Table 13.

Table 13. Consumption of locally caught fatty fish with respect to miscarriages and stillbirths.

	No n (%)	High n (%)	OR (95% CI) for high vs. low
Wives			
East coast	7 (5)	11 (2)	0.62 (0.23-1.66)
West coast	6 (4)	46 (8)	1.26 (0.51-3.07)
Sisters			
East coast	8 (1)	10 (4)	0.83 (0.32-2.16)
West coast	35 (6)	7 (3)	0.97 (0.42-2.27)

CB-153

Among the east coast fishermen's sisters, the women whose first planned pregnancy ended in a miscarriage or stillbirth had lower estimated past CB-153 concentrations than those who had live births (126 vs. 218 ng/g lipid; p=0.004; Paper V; Figure 14). The results were similar when the fishermen's wives were included (146 vs. 227 ng/g lipid; p=0.005). However, no effect was found when the analysis was performed on the east coast fishermen's wives only (207 vs. 257 ng/g lipid; p=0.5; previously unpublished data).

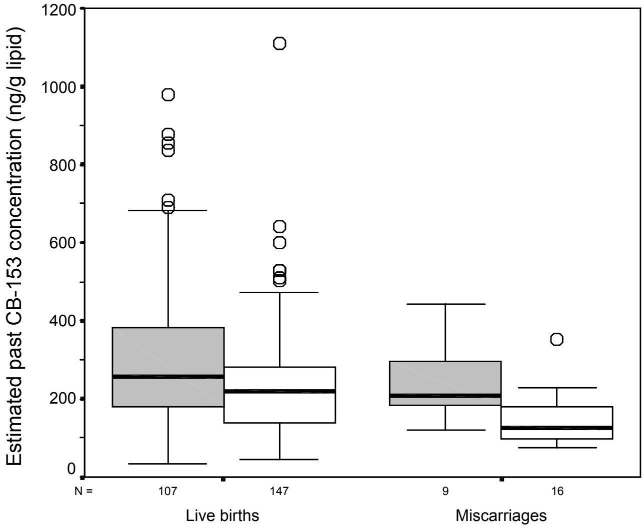


Figure 14. Estimated past CB-153 concentrations in east coast fishermen's wives (grey boxes) and fishermen's sister (white boxes) with live births and miscarriages/stillbirths.

Discussion

MARKERS OF FERTILITY

Time to pregnancy

Time to pregnancy is a measure that takes into account effects on the male and female reproductive capacity, as well as the early survival of the foetus. It has been found to be a useful tool for assessing reproductive effects of exposures in general (51) and environmental exposure in particular (15). Furthermore, several studies have shown that time to pregnancy can be assessed using a self-administered questionnaire (14, 54, 97).

The ideal way to study time to pregnancy is to follow a group of women in a prospective study. A retrospective study of time to pregnancy relies on the woman's ability to accurately remember the circumstances surrounding her pregnancies, and in particular, the length of the time she had to try to achieve conception. In the present studies, the recall times are quite long: for the fishermen's wives the median recall time was 20 (range 0-37) years and for the fishermen's sisters the median recall time was 18 (0-38) years. However, studies have found that recall of time to pregnancy is remarkably good at a group level, even at recall times of 14 years and longer (53, 54).

When assessing time to pregnancy retrospectively, women who never conceived are lost since they cannot supply a time to pregnancy. However, both the fishermen's wives and sisters were asked if they at some point in their life unsuccessfully tried to conceive for a consecutive period of at least 12 months. These women were included in subfertility-analyses, and also in a time to pregnancy-analysis where it was assumed that their time to pregnancy was longer than 12 months (i.e. censored at 12 months). This did not change the effect estimate for the fishermen's sisters cohort comparison, but increased the effect of east coast cohort affiliation among the fishermen's wives, especially for the heavily smoking women. This was to be expected, since these women also had an increased subfertility risk.

The original idea with the questionnaire that was sent to the fishermen's wives was to collect information on all pregnancies of each woman. However, there was no information on the maximum number of pregnancies prior to the study, and it was decided that a restriction should be made to each woman's five first pregnancies. Due to reasons previously mentioned, the first planned pregnancy was then chosen for analysis of time to pregnancy and miscarriages. Because of the low response rate from the fishermen's wives questionnaire, and the fact that only information on the first planned pregnancy was actually used, a shorter questionnaire was sent to the fishermen's sisters. However, when analysing all pregnancies from the fishermen's wives, the time to pregnancy results were similar to those obtained when only the first planned pregnancy was included in the analysis. Furthermore, among the fishermen's sisters, the risk of ever having experienced a miscarriage or a stillbirth was similar to the risk of experiencing a miscarriage in the first planned pregnancy. Thus, it seems reasonable to assume that the information collected in the short questionnaire was sufficient.

The time to pregnancy was assessed differently for the fishermen's wives and sisters, in that it was possible for the fishermen's wives to claim a zero month time to pregnancy whereas the shortest possible time to pregnancy for the fishermen's sisters was one month. Thus, it may be argued that no joint analysis should be performed on the two datasets. However, letting the zero-months times to pregnancy be represented as one-month times to pregnancy did not change the effect estimate and neither did excluding the zero month times to pregnancy. Hence, the analyses of the joint material should not be invalid.

In the analyses of time to pregnancy each woman's first planned pregnancy was used. Thus, not all pregnancies included were of parity one. However, parity was considered as a potential confounder in all analyses. The reason for selecting the first *planned* pregnancy rather than the first pregnancy was to increase the number of observations included in the analyses. However, subgroup analyses show that the effect estimates are similar when analysing all first planned pregnancies, and when including only first parity pregnancies. Using the first planned pregnancy requires longer recall than the latest planned pregnancy, which normally can be used in time to pregnancy analyses. However, in this specific case, where the body burden of exposure decreases during lactation, using later pregnancies may result in the loss of the most highly exposed women.

Subfertility

The fraction of subfertile women was higher among the fishermen's wives than among the fishermen's sisters. However, the definition of subfertility differed between the fishermen's wives and sisters, in that wives who had given a time to pregnancy longer than 12 months in at least one of their five first pregnancies were also considered subfertile. Since the fishermen's sisters were asked only about their first planned pregnancy, it was not possible to use the same definition for these women. When using the same definition of subfertility for the fishermen's wives as for the fishermen's sisters, the risk associated with east coast cohort affiliation was somewhat increased. Thus, if any bias has been introduced it is most likely to have resulted in an overestimation of the true risk among the fishermen's sisters.

CONFOUNDERS AND EFFECT MODIFIERS

Cohort comparability

Several socio-economic factors, such as alcohol (50) and caffeine consumption (22, 34, 87) as well as smoking (21, 34) have been suggested to affect fertility, as well as the risk of giving birth to a stillborn infant (88). The reason for comparing east coast fishermen's families to west coast fishermen's families was that they, on a group level, were assumed to be similar with respect to socio-economic factors. This was found to be true among the women who responded to the questionnaires, with the exception of smoking habits, which was found to be an effect modifier among the fishermen's wives.

Smoking

In the studies on fishermen's wives and sisters there is no consistent effect of female consumption of POC-contaminated Baltic Sea fatty fish on time to pregnancy. There was a prolonged time to pregnancy among heavily smoking east coast fishermen's wives compared to heavily smoking west coast ones. This could be explained if the heavily smoking east coast women were more exposed to POC than the non/light smoking east coast women. However, in the subgroup of women who supplied blood for analysis of CB-153 concentration, the heavily smoking women had lower median concentration than the non/light smoking women (Figure 12).

Among the east coast fishermen's wives and the west coast fishermen's sisters, smoking more than 10 cigarettes per day was found to increase the time to pregnancy compared to women who smoked less than 10 cigarettes per day. The same effect was not found for the west coast wives and east coast sisters. Smoking has been described to prolong the time to pregnancy by about 25% to 55% (21, 34, 50). Thus, the unexpected results are observed among the west coast wives and east coast sisters. Among the fishermen's sisters who smoked more than 10 cigarettes per day, the number of cigarettes smoked in the east and west coast cohort were similar (median 15 and 12.5 cigarettes per day). Among the fishermen's wives who smoked more than 10 cigarettes per day, the proportion of women who smoked more than 20 cigarettes per day were similar in both cohorts (14% and 15% in the east and west coast cohort, respectively). Thus, the lack of an effect of smoking in two of the cohorts cannot be explained by residual confounding.

Beneficial constituents in fatty fish

A higher percentage of the fishermen's sisters than of the fishermen's wives had grown up in a fisherman's family and/or a fishing village. However, the fishermen's wives reported a higher current fish consumption than the fishermen's sisters. Hence, these two groups of women have probably been exposed to POC through the consumption of Baltic Sea fatty fish at different stages in their lives. The results found for both the fishermen's sisters and wives indicated a protective, rather than hazardous, effect of fatty fish consumption. However, the studies were designed to investigate whether the consumption of fatty fish lead to unwanted reproductive effect. Positive effects of other constituents (e.g. polyunsaturated fatty acids), their possible protective mechanisms, and optimal time window for exposure, can therefore not be determined. Other studies have found positive effects of consumption of non-contaminated fatty fish on reproductive outcomes such as birth weight (65-67) and duration of gestation (43, 65, 67). However, the effect on time to pregnancy has not been evaluated.

BIAS

Selection bias and recall bias

With the fishermen's wives questionnaire, an introductory letter was sent in which the purpose of the study was stated to be an investigation of fertility in relation to intake of fatty fish contaminated with POC. It was, however, not clarified that it was the fish caught on the east coast of Sweden that was contaminated. In a study carried out the year before, only 5% of the east coast fishermen's wives were aware of the potential hazards of Baltic Sea fatty fish contaminated with POC (79). Thus, recall bias should be only a minor issue in the fishermen's wives analyses. However, when approaching the fishermen's sisters the circumstances were somewhat different. The response rate to the fishermen's wives questionnaire was very low, and in an effort to achieve a higher rate among the fishermen's sisters they were clearly informed about the aim of the study, and the results from the previous study on fishermen's wives. However, these results had already been presented in Swedish radio and newspapers. Furthermore, it must be considered that the fishermen's wives are the sisters-in-law of the fishermen's sisters and it is not unlikely that the study on fishermen's wives had already been discussed within the fishermen's families. Nevertheless, the fact that the fishermen's sisters were informed about the purpose of the study may have introduced recall bias, as well as selection bias, to the fishermen's sister study. The selection bias should be of minor concern, since the non-respondent analyses found similar differences between responders and non-responders from the east and west coast. However, the recall bias may have caused an overestimated effect of cohort affiliation.

Reduced fecundability does not necessarily translate into a reduced number of children (23). Furthermore, the respondents and non-respondents to the fishermen's sisters questionnaire differed in the percentage of women never having given birth. Thus, even though the percentage who had given birth at least once did not differ among the non-respondents and respondents to the fishermen's wives questionnaire, it can not be ruled out that a selection bias with respect to fertility is present in both the fishermen's wives and fishermen's sisters studies. However, for both the fishermen's wives and sisters, the difference between respondents and non-respondents were similar between cohorts and thus selection bias with respect to fertility should not be an issue of concern in the cohort comparisons.

Misclassification of exposure

The cohort affiliation of both the fishermen's wives and sisters were decided based upon the residence of the husband/brother. For the fishermen's wives it is reasonable to assume that the residence of the husband is the same as the residence of the wife. This is not necessarily true for the fishermen's sisters. However, when giving birth in 1973-75, 90% of the east and 97% of the west coast fishermen's sisters resided on the same coast as their brother. Out of the 34% of fishermen's sisters with known place of residence, 85% of the east and 88% of the west coast women resided on the same coast as their brother. Thus, the fishermen's families are quite stable with respect to their residence, and the fact that the current residence of their brother determined the cohort affiliation of the fishermen's sisters should not have resulted in any major misclassification.

Both questionnaires were designed primarily to study time to pregnancy, and for some factors the information collected was therefore focused on the time before conception, whereas the relevant time period when investigating miscarriage risk is early pregnancy. Therefore, some of the exposure measures employed must therefore be considered as proxies. However, there is no reason to believe that possible changes in these potential confounders would differ between cohorts.

The CB-153 concentrations for the fishermen's wives were analysed from plasma, whereas the concentrations for the fishermen's sisters were analysed from blood. Furthermore, the concentrations were not determined at the same laboratory. However, when determining concentrations of CB-153 it is irrelevant whether plasma or serum is used in the analyses (Åke Bergman, personal communication). Moreover, 25 of the samples from the fishermen's wives were analysed by both laboratories, and the inter-laboratory correlation was very high (r_S=0.99).

Due to small sample volumes it was not possible to determine the lipid concentration in the rubella serum samples. For the model validation comparisons were therefore made between fresh weight concentrations. The model was then, however, applied on lipid adjusted CB-153 concentrations. As a high correlation was observed between the fresh weight and lipid-adjusted CB-153 concentrations (r_S=0.91), this may only have had a minor effect on the estimates.

The model for estimating past CB-153 concentrations assumes a stable background exposure and a constant intake of Baltic Sea fatty fish. It was found for the fishermen's wives that only a few had a probable high consumption of fatty fish during childhood and adolescence, whereas their consumption during adult life was relatively high. The fishermen's sisters, on the other hand, were more likely to have had a high fatty fish consumption during childhood and adolescence, but did not have as high consumption as adults. The model for estimating past CB-153 was originally designed for the fishermen's wives, and it is reasonable to assume that the assumption about a constant intake of Baltic Sea fatty fish is not violated for these women. This is not necessarily true for the fishermen's sisters. However, the validation shows that the estimated past concentrations agree better with actual past values than do the current concentrations. The model adjusts for an environmental decrease of CB-153, decrease of body burden during lactation, and the biological half-life of CB-153. The true values for these parameters are not known, and it is possible that the decrease in environmental CB-153 assumed in the model reflects the decrease in intake of CB-153 due to environmental changes as well as changes in fish consumption and background exposure.

It was assumed that no decrease in environmental CB-153 was present before 1976, which is a reasonable assumption since the concentrations of PCB did not start to decrease until the use of PCB was banned. However, environmental CB-153 may have *increased* before the ban in 1972. Almost 25% of the women for whom concentrations of CB-153 was available conceived in their first planned pregnancy before 1972, and a further 25% conceived between 1972 and 1976. However, in the validation of the model for estimation of past concentrations of CB-153, only 4 women conceived before 1976, and none of the women conceived before 1972. Thus, it is possible that the model is unstable for conceptions before 1972 or 1976, which may result in an underestimation of the true past concentrations of CB-153.

Misclassification of outcome

Of 183 east coast fishermen's sister who had previously provided a time to pregnancy, 14 stated that they had never had a planned pregnancy when they were asked to participate in a second study (Paper V). Of these women, 9 had given a one-month time to pregnancy in the original questionnaire, which is to be expected if the pregnancy is not planned. Three women had given a time to pregnancy exceeding 12 month. Two of these became pregnant after medical treatment, and it can be argued that they did not consider this a "normal" planned pregnancy, thus being unwilling to state a time to pregnancy the second time asked. The third woman had given the time between her two pregnancies (36 months) as the time to pregnancy. There is no reason to believe that east coast women to a higher degree than west coast women would report unplanned pregnancies as planned pregnancies with relatively long times to pregnancy. Thus, no bias should have been introduced by this misclassification of time to pregnancy. In support of this, when excluding all women who stated that their time to pregnancy was zero or one month, the effect estimates remained almost unchanged compared to when all times to pregnancy were included.

COMPARABILITY WITH OTHER STUDIES

Animal data

In studies on Rhesus monkeys, lowered offspring birth weight, increased risk of spontaneous abortions, and affected menstrual cycle seemed to occur at lower dose levels than what was needed to produce lowered conception rate (cf Figure 5). In previous studies on fishermen's wives and sisters, an increased risk of giving birth to a low birth weight infant was found (77-80). However, in the present studies there is no support for an increased risk of spontaneous abortions or prolonged time to pregnancy among the east coast fishermen's wives and sisters. After 25 months of continuous oral exposure to Aroclor 1254, 90% of 80 female Rhesus monkeys had reached a qualitative pharmacokinetic steady state of 9-106 μg/g lipid, depending on the dose of Aroclor received (8). Thus, the Rhesus monkeys with the lowest exposure still achieved more than a ten-fold body burden as did the most highly exposed fishermen's wives and sisters. It has been suggested that in humans, the developing embryo/foetus is more sensitive than the mother (70). This seems to hold true also for the fishermen's wives and sisters where a lowered infant birth weight but no effect on female fertility was found.

Other epidemiological studies

The blood from the east coast fishermen's wives and sisters included in the present study was analysed for CB-153 only. However, from a study by Hagmar *et al*. (47), the total PCB concentration in plasma for some of the east coast fishermen's wives can be crudely estimated. The crude estimate was 4.2 ng/g, which is slightly higher than what was found among women from the Dutch (0.7 ng/g) (69) and German (2 ng/g) (40) general populations, but similar to those concentrations found in the Michigan Maternal Infant Cohort (5.5 ng/g) (39). It has also previously been shown that the estimated current dietary exposure to POC through fish consumption has been found to be comparable between groups of high consumers from the Baltic Sea and the Lake Ontario regions (2). The results from the time to pregnancy-analyses for the fishermen's wives and sisters are similar to studies performed on populations from the Great Lakes area, where one study found a decreased fertility for women with a high consumption of Lake Ontario fish (30), whereas other studies failed to show the same effect (29, 33).

Increased concentrations of CB-153 did not prolong the time to pregnancy for the fishermen's wives and sisters. No other study has investigated the relation between a biomarker for PCB and fertility. However, attempts have been made to estimate lifetime PCB exposure, but no evidence of an effect of this exposure was found on fertility (30).

There was no increased miscarriage risk for the east coast fishermen's wives and sisters, which is consistent with results from studies on populations in the Great Lakes area (36, 61).

General conclusions

The current study provides no evidence for a decreased fertility, measured as a prolonged time to pregnancy, among women with a high consumption of Baltic Sea fatty fish, neither during childhood and adolescence, nor during adult life. Furthermore, there is no consistent evidence of a decreased fertility, measured as ever unsuccessfully having tried to conceive for a consecutive period of 12 months or more, among these women. The results with respect to time to pregnancy among exposed women who are also heavy smokers are ambiguous.

A high intake of fatty Baltic Sea fish during childhood and adolescence, as well as during adult life, seemed to decrease, rather than increase, the miscarriage risk.

There were indications that women with high estimated past CB-153 concentrations tended to experience a shorter time to pregnancy than women with low estimated past CB-153 concentrations. These results were, however, not consistent for both study groups (fishermen's wives and fishermen's sisters). Among the fishermen's sisters, women with miscarriages had lower estimated past CB-153 concentrations than those with live births. This was not confirmed by the analyses concerning fishermen's wives.

In conclusion, a couple's fertility does not seem to be affected by consumption of fatty fish from the Baltic Sea on the part of the woman. Furthermore, at the levels of CB-153 found in consumers of Baltic Sea fish, high CB-153 concentrations does not seem to infer decreased fertility.

Further research

Only a few studies have investigated the effect of paternal exposure to POC through consumption of contaminated fatty fish on reproductive outcomes such as time to pregnancy 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 (33), whereas paternal Lake Ontario fish consumption did not show any increase in risk (28). A study on semen quality among fishermen from the Swedish east coast is currently underway. Fishermen whose wives participated in the time to pregnancy cohort study will be able to constitute a basis for a study investigating the effect of paternal exposure on time to pregnancy and miscarriages.

Animal studies have found that lowered offspring birth weight, increased risk of miscarriages, and affected menstrual cycle occur at similar dose levels of PCB. Furthermore, among the women in the New York State Angler Cohort, a decrease in menstrual cycle length was found (60). Thus, with birth weight and miscarriages already studied among the fishermen's wives and sisters, possible effects on the menstrual cycle remains to be investigated.

Within the east coast cohorts there were indications of a protective, rather than a hazardous, effect of exposure. Other studies have found a positive correlation between consumption of non-contaminated fish and reproductive outcomes such as gestational length and birth weight (43, 65-67). It would therefore be of interest to investigate whether consumption of fatty fish caught on the west coast of Sweden is associated with fertility.

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Populärvetenskaplig sammanfattning på svenska

Polyklorerade bifenyler (PCB) är ett samlingsnamn för ett antal kemiska föreningar. De utmärker sig genom att de har en god elektrisk isoleringsförmåga och tålighet för höga temperaturer, och de har därför använts i bl.a. transformatorer och kondensatorer men även som mjukgörare i plaster och färger. Sedan det visat sig att exponering för PCB innebar allvarliga hälsoeffekter, infördes i Sverige 1972 ett förbud mot användning av ämnet. Emellertid är PCB mycket stabila kemikalier, även i biologiska system, vilket har medfört att PCB har stor spridning i hela vår omgivning. Detta beror på att kemikalien är fettlöslig och metaboliseras långsamt. Den lagras därmed i fettet hos både människor och djur och transporteras uppåt i näringskedjan. CB-153 (2,2',4,4',5,5'-hexaklorobifenyl) är en av 209 varianter av PCB. Forskning har visat att koncentrationen av CB-153 i plasma och blod ger en mycket god bild av en människas koncentration av total PCB, och man kan därför använda CB-153 koncentration som ett mått på exponering för total PCB.

Djurstudier har visat att exponering för PCB kan ge effekter bl.a på menstruationscykeln och nedsatt fertilitet hos honor, men också att avkomma till exponerade honor har en lägre födelsevikt. Hos människor kan fertilitet mätas genom att undersöka *väntetid till graviditet*, d.v.s. den tid som passerar från den tidpunkt då ett par slutar använda preventivmedel till den tidpunkt då kvinnan blir gravid. Detta kan mätas endast om graviditeten var planerad. Genom att undersöka denna väntetid till graviditet täcker man in effekter på såväl mannen och kvinnan som fostret fram till dess graviditeten fastställs. När graviditeten väl är fastställd kan man undersöka fostrets överlevnad genom att titta på missfallsrisk.

I Sverige är konsumtion av fet fisk (t.ex. lax och sill) från Östersjön en av de främsta källorna till exponering för PCB. Det har tidigare visats att svenska yrkesfiskare och deras familjer i genomsnitt äter mer fisk är vad folk i allmänhet gör. Vidare har man funnit att yrkesfiskarna och deras syskon växt upp i fiskarfamiljer och fiskelägen relativt ofta, något som kan antas ha lett till en hög konsumtion av fisk även under uppväxten. För att studera eventuella hälsoeffekter av exponering för PCB genom konsumtion av fet fisk från Östersjön har därför grupper av yrkesfiskare från Sveriges ostkust identifierats. Genom matchning med olika register har grupper av hustrur och f.d. hustrur till dessa yrkesfiskare (fiskarhustrur), samt grupper av systrar och halv-systrar (fiskarsystrar) kunnat identifierats. Som jämförelsepopulation (icke exponerad population) har motsvarande grupper identifierats bland yrkesfiskare på svenska västkusten.

Enkäter skickades till fiskarhustrur och fiskarsystrar för att samla in information om deras *första planerade graviditet*. Förutom väntetid till graviditet ställdes även frågor om graviditetens utfall för att kunna beräkna missfallsrisken på gruppnivå. Dessutom frågades efter andra faktorer som också kan misstänkas påverka fruktsamhet, som t.ex. rökvanor och arbetstider. Svarsfrekvensen i de båda grupperna låg strax under 60 % (1090 väst- och 505 ostkustfiskarhustrur, samt 1103 väst- och 709 ostkustfiskarsystrar). För 121 ostkustfiskarhustrur och 165 ostkustfiskarsystrar analyserades blodprov för att bestämma koncentrationen av CB-153. Denna koncentration användes sedan som ett mått på exponering för PCB.

Fiskarhustrurna från ostkusten hade en längre väntetid till graviditet jämfört med fiskarhustrurna från västkusten (se tabell). Det visade sig att denna effekt berodde på att de ostkustkvinnor som rökte mer än tio cigaretter per dag hade en fördubblad väntetid till graviditet jämfört med de västkustkvinnor som rökte mer än tio cigaretter per dag. Bland dessa kvinnor var det också fler som vid något tillfälle i sitt liv hade en väntetid till graviditet som överskred 12 månader (34 % på västkusten jämfört med 54 % på ostkusten). Det syntes ingen skillnad mellan de ostkustkvinnor som rökte mindre än tio cigaretter per dag och de västkustkvinnor som rökte mindre än tio cigaretter per dag. Bland fiskarsystrarna kunde motsvarande förlängda väntetid till graviditet inte påvisas.

Tabell. Genomsnittligt (median) antal månaders väntetid till graviditet, samt gränserna för den "mellersta" halvan (25:e och 75:e percentilen).

	Ostkust		Västkust	
Fiskarhustrur	3	(1 – 6)	2	(1 – 6)
0-9 cigaretter/dag	2	(1 – 5)	2	(1 – 6)
≥ 10 cigaretter/dag	4	(1 – 12)	2	(1 – 6)
Fiskarsystrar	2	(1 – 5)	2	(1 – 4.5)
0-9 cigaretter/dag	2	(1 – 6)	2	(1 – 4)
≥ 10 cigaretter/dag	1.5	(1 – 5)	2	(1 – 7)

Vid analyser inom ostkustgrupperna (fiskarhustrur såväl som fiskarsystrar) förelåg en förkortad, snarare än förlängd, väntetid till graviditet för kvinnor som växt upp i en fiskarfamilj eller fiskeläge, och för kvinnor som idag äter mycket ostkustfisk. Dessa skillnader var dock inte statistiskt säkerställda. Det fanns ingen skillnad i väntetid till graviditet mellan ostkustfiskarhustrur med hög och låg koncentration av CB-153. Dock fanns en *förkortad* väntetid till graviditet hos ostkustfiskarsystrar med medel och hög koncentration av CB-153, så till vida att kvinnor med i "medelgruppen" hade 62 procents kortare tid till graviditet, och kvinnor i "höggruppen" hade 82 procents kortare tid till graviditet än vad "låggruppen" hade.

Ostkustkvinnorna hade inte högre risk för missfall än västkustkvinnorna. Inte heller förelåg en ökad risk för de ostkustkvinnor som var uppväxta i fiskarfamilj eller fiskeläge, eller som idag har en hög konsumtion av fet ostkustfisk. Vidare hade ostkustkvinnor som haft missfall eller fött dödfödda barn inte högre koncentrationer av CB-153 än de kvinnor som fött levande barn.

Sammanfattningsvis ger dessa studier inget belägg för att kvinnans exponering för PCB genom konsumtion av fet östersjöfisk påverkar fruktsamheten. Vidare syns ingen påverkan av fruktsamheten av de koncentrationer av CB-153 som finns hos storkonsumenter av östersjöfisk.

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Appendix

In order to establish a theoretical population that resembled the fishermen's sisters and fishermen's wives with respect to their time to pregnancy, the percentage of women with different times to pregnancy were calculated (Table 14). The distribution of time to pregnancy in the theoretical population was then chosen based upon these two distributions. However, when information on time to pregnancy is collected retrospectively, as was the case with the fishermen's wives and fishermen's sisters, it is likely that a digit preference is present. Therefore the theoretical population was designed as a non-increasing series. From the theoretical population, 100 times to pregnancy were then randomised. The distribution of these is shown in Table 14.

Table 14. Time to pregnancy among fishermen's wives and fishermen's sisters, as well as an assumed distribution of time to pregnancy in a population that is similar to the fishermen's families.

Time to pregnancy (months)	Fishermen's wives	Fishermen's sisters	Theoretical population	Simulated population
1	37%	45%	42%	39
2	15%	17%	16%	16
3	11%	8%	10%	14
4	6%	4%	5%	5
5	3%	3%	4%	5
6	8%	4%	4%	3
7	1%	2%	3%	2
8	1%	2%	2%	2
9	1%	1%	1%	1
10	1%	1%	1%	2
11	1%	1%	1%	1
12	5%	2%	1%	2
> 12	10%	11%	10%	8

In order to calculate Success Odds Ratios (SORs) and Success Rate Ratios (SuRRs), five additional populations were determined using five distribution shift factors (0.50, 0.75, 1.25, 1.50 and 2.00). For each of these additional populations a theoretical distribution was calculated by dividing the fraction of women who got pregnant each month during the first year by the distribution shift factor (Table 16).

Table 15. Median time to pregnancy in six randomised populations.

	Distribution shift factor					
	0.50	0.75	1.00	1.25	1.50	2.00
Median time to pregnancy (months)	1.0	1.0	2.0	3.5	6.0	13.0

Table 16. Distributions of times to pregnancy in six randomised populations.

Time to pregnancy (months)	Distribution shift factor 0.50	0.75	1.00	1.25	1.50	2.00
1	84%	56%	39%	25%	27%	14%
2	16%	12%	16%	19%	9%	12%
3		19%	14%	6%	6%	3%
4		9%	5%	2%	3%	3%
5		4%	5%	4%	3%	3%
6			3%	6%	4%	2%
7			2%	6%	3%	3%
8			2%		1%	1%
9			1%	1%		
10			2%	1%		2%
11			1%			
12			2%		2%	
>12			8%	30%	42%	57%