This is to certify that the
thesis entitled

IN UTERO EXPOSURE TO PCB AND DDE AND THEIR INFLUENCE ON MALE FECUNDABILITY

presented by

JYOTSNA MUTTINENI

has been accepted towards fulfillment
of the requirements for the

Master of Science	degree in	Epidemiology

Major Professor's Signature

12/1/03

Date

IN UTERO EXPOSURE TO PCB AND DDE AND THEIR INFLUENCE ON MALE FECUNDABILITY

By

Jyotsna Muttineni

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Epidemiology

2004

ABSTRACT

IN UTERO EXPOSURE TO PCB AND DDE AND THEIR INFLUENCE ON MALE FECUNDABILITY

By

Jyotsna Muttineni

To study the influence of *in utero* exposure to polychlorinated biphenyls (PCB) and dichlorodiphenyldichloroethylene (DDE) on the fecundability of male offspring we conducted a in two generations of the Fisheater Family Cohort. The serum PCB and DDE concentrations were determined in the parent generation during three surveys conducted between 1973-1991. We have serum PCB and DDE determinations from 391 women in the parent generation of which 259 provided offspring information. Of the 202 eligible (20-50 years of age) male offspring 103 (50.9%) agreed to participate. We collected information regarding the time intervals of unprotected intercourse (TUI) through telephone interviews. The offspring had 172 TUI, 160 ended in pregnancy. Fecundability ratio (FR) was estimated using 'proportional hazards regression model' (PHREG). Exposure to PCB (1-<5 μg/ dL) was associated with a high FR (2.7, 95%CI 1.35-5.39). Alcohol consumption and frequency of sexual intercourse showed a positive association with FR. This is the second study analyzing the association between PCB, DDE on fecundability.

DEDICATION

I am dedicating this thesis to my parents

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to Dr. Karmaus, for all the encouragement and guidance he provided me during these two years. I would also like to thank him and Dr. Karna for providing an opportunity to work with them as a graduate assistant. I would like to thank Dr. K. Chou and Dr. P. Pathak for their expert advice. My friend and my roommate for two years Spandana without whom this completion would not have been possible. Oana, Ting, Kevin, Ali, and Susan – Thank you for all the valuable suggestions and best of luck in all your future endeavors.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abbreviations

PCB Polychlorinated biphenyls

DDE Dichlorodiphenyldichloroethylene

DDT Dichlorodiphenyltrichloroethane

TTP Time to pregnancy

TUI Time of unprotected intercourse

PUNP Periods of unprotected intercourse

PE Phthlate esters

CHAPTER 1

Introduction

There have been a number of reports concerning animal and human fecundity in the past decade. (1) These studies focused on the various factors influencing fecundity, one of them being environmental pollutants. (2, 3) The present study examines the influence of *in utero* exposure to polychlorinated biphenyls (PCB) and dichlorodiphenyldichloroethylene (DDE) a metabolite of dichlorodiphenyltrichloroethane (DDT) on male fertility. Consumption of fish from the Great Lakes is a major source of exposure to PCBs, DDT and their metabolites. (4) Placental transfer of these compounds is well documented. (5) Serum levels of maternal PCB, DDT and DDE in Michigan Anglers are used as a measure of exposure to PCB and DDE *in utero*. I will analyze the male offspring of a cohort of Michigan Anglers. Time to Pregnancy (TTP) is used as a measure of fecundity in the offspring (second generation). This study describes the exposure scenario, characteristics of PCB, DDE and provides evidence of the influence
of these substances on fertility in humans and animals in various studies.

Exposure in the Great Lakes region to PCBs and DDE

The Great Lakes constitute $1/5^{th}$ of the major fresh water reservoirs on Earth. The Great Lakes watershed has been a major agricultural, industrial, and recreational center in North America. (6) The Great Lakes are open to the oceans through St. Lawrence River, however they behave more like a closed system. Any disturbance in the ecosystem of

the Great Lakes becomes permanent or takes long duration to be free of it. The water exchange rates in the Great Lakes are very low, taking three years for exchange of water in Lake Erie and 173 years for Lake Superior. (7, 8)

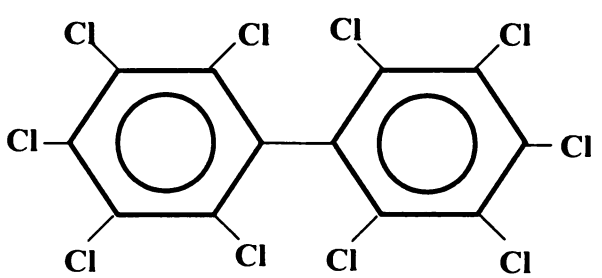
The Great Lakes receive discharges from the industries, agricultural areas and cities surrounding them. These discharges are the major source of the various chemicals seen in the Great Lakes. The Virtual Task Force of the International Joint Commission identified 11 persistent toxic substances in the Great Lakes, of which six of them PCB, DDT, dieldrin, mirex, toxaphene, and alkylated lead are now banned from manufacturing and sale. (9)

The concentrations at which each of these chemicals are found and their influence on the ecosystem and the humans living in the watershed is of concern. Of the various pollutants organochlorines are the most persistent compounds which bioaccumulate in living organisms. As a result of biomagnification concentrations of these compounds increase in the trophic levels of food chain and humans being at the end of the food chain are exposed to high concentrations. (10)

Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls are organochlorine compounds with an empirical formula $C_{12}H_{10-n}Cl_n$. (Figure 1)

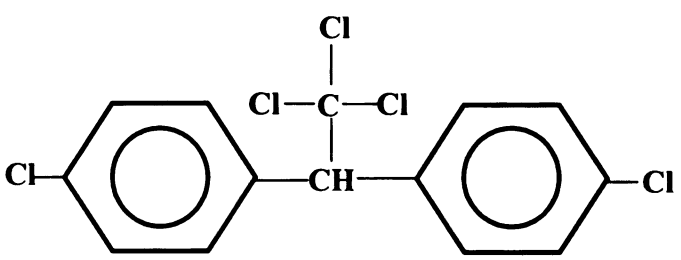
Figure 1. Chemical structure of PCB (Number of chlorines can vary)



PCBs were first synthesized in 1881 and were used extensively since the 1930's in United States. PCBs had different chlorine content depending on the duration of chlorination when synthesized. They do not occur naturally and were never to enter the environment; however accidental leaks, accidents in transport and leakage from hazardous waste sites and illegal dumping have lead to their existence in the environment. PCBs are lipophilic with long half-life of up to 70 years. (11) Of the Great Lakes, Lake Michigan contains the largest amount of PCB residue. (10)

Dichlorodiphenyltrichloroethane (DDT)

Figure 2. Chemical structure of DDT



DDT (Figure 2) was a major insecticide after World War II. DDT is metabolized in living organisms to DDE, which has a half-life of about eight years and it bioaccumulates in nature. Use of DDT was banned in the USA in 1977. However, its residues are still found in humans and animals. (12) The properties of DDT and its metabolites enable them to be taken up readily by organisms. High lipid solubility and low water solubility lead to the retention of DDT and its metabolites in fatty tissue. (13)

Fishing is a major recreational activity and a source of livelihood for many residents around Lake Michigan; and these recreational anglers consume fish more than

three times the national average (10) (National average = 38g/ day) Studies have shown that recreational anglers and fisheaters in Michigan have higher serum concentrations of DDE (dichloro diphenyl ethene) and PCBs. (14-16)

The effects of PCBs and DDE on male fertility has been observed through many studies in humans and animals using serum/ seminal PCB/DDE levels as a measure of exposure and seminal analysis as the outcome.

Studies on the effects of PCBs and DDE on fertility in men

Seminal analysis can be used as a surrogate measure of male fecundity in clinical andrology, reproductive toxicology, epidemiology and risk assessment. Sperm count, motility, velocity, linearity or lateral head displacement and penetration capacity using hamster eggs constitute seminal analysis. The sperm concentration, proportion of normal sperm, and morphological anomalies per abnormal spermatozoon influenced TTP (time to pregnancy) in humans. (17) Fecundability in men increases from zero to 20% with increasing sperm count up to 40million/ml but above this concentration sperm count and fertility do not show any increased fecundability. This shows that a substantial change in sperm count may not be associated with a severe reduction in the fecundability. (18)

Seven human studies have been conducted to demonstrate the influence of exposure to PCB and DDE on male fertility, which used various markers of exposure to PCBs in humans to assess male fertility. However, only one study has been done to my knowledge on the influence of prenatal exposure to PCB and subsequent male fertility. (19) A large scale poisoning of PCB occurred in Taiwan in 1979 and the male offspring who were prenatally exposed were analyzed and found to have increased abnormal

morphology, and reduced motility of the sperm. The sperm analyzed in this study also showed decreased capacity to penetrate hamster oocytes. However, the second generation offspring are very young (< 20 years).

In a study to assess the exposure to organochlorines due to fish consumption from the Great Lakes Courval et al. have shown an association between consumption of sport caught fish from the Great Lakes and conception delay in men. (20) This study did not determine organochlorines but used consumption of fish as an indirect measure.

A study on 29 males in Israel at an infertility clinic, each of them with at least a five year history of infertility showed that spermatozoa motility was negatively correlated with residues of organochlorine compounds (DDT and its metabolites and PCB). (21) However there was no correlation with spermatozoa count and morphology.

Bush et al. determined PCB concentration in sperm from 170 seminal samples and found a positive correlation between sperm count and motility. (22) The concentration of PCB congeners in seminal samples was inversely related with sperm motility.

Another study found that seminal plasma levels of PCBs were negatively correlated with sperm count. This study also found that the concentration of PCBs increases with age whereas p, p'DDE and DDT did not show a similar relationship. (23)

A Flemish study in Antwerp and Peer (Belgium) showed that men in Peer had very low normal sperm morphology, with very low values of testosterone and spermatozoa. (24) This study did not estimate the organochlorine exposure in these men. However, women residing in Peer have higher serum concentrations of DDT, DDE, lindane and pentachlorophenol compared to Antwerp.

In a pilot study done by Hauser et al. on men undergoing medical evaluation due to the couple being unable to conceive the concentrations of PCB congeners and p'p' DDE in the serum were found to be higher in men whose semen showed a below normal motility of sperm. (25) This pilot study shows that a negative association exists between concentration of PCB, p' p' DDE and sperm motility, morphology and concentration.

A study was done in India to assess the role of PCB and phthlate esters (PE) in the deterioration of semen parameters in infertile men (n=21) without an obvious etiology, and in 32 controls. (26) The study found a negative correlation between seminal PCB concentrations and ejaculate volume, motility, sperm vitality and osmoregulatory capacity. However, they did not find a significant correlation between PCBs and sperm count, morphology with head defects. This study also found that the PCB and PE concentrations were highest in infertile urban fisheaters followed by infertile rural fisheaters followed by vegetarians respectively.

Studies on the influence of PCBs and DDE on male fertility in animals

Studies have been done in animals to determine the influence of PCBs on male fertility. Some of these studies used number of litters and litter size, and others used seminal analysis as an indicator of fertility.

One such study was done on Rhesus monkeys. (27) There are four two-generational studies done to study the influence of PCB on male fertility in the two generations. (28-31) Two studies have been done in rats to assess the influence of DDT on male fertility. (32-33)

Adult male Rhesus monkeys (n=4) were given a diet containing 5.0 ppm PCB for 18 months and they found a marked decrease in the sperm counts and failed to impregnate females after repeated breedings. (27) Testicular biopsy revealed hypocellularity of seminiferous tubules and absence of spermatozoa. A second biopsy taken one year after the animals were removed from the experimental diet showed a complete recovery of spermatogenesis.

A multi-generational study was done by Linder et al. in Sherman rats exposed to various levels of PCB (Aroclor 1254, 1260) (0, 20, 100 ppm) prenatally and in their diet. (28) This study showed that increased Aroclor 1254 exposure decreased the number of litters (18, 17, 7), and also decreased the litter sizes significantly (12.3, 10.4, 9.5). However when the Sherman rats were exposed to Aroclor 1260 there was not a significant decline in the number of litters (18, 19, 20) and litter size (10.9, 11.3, 10.1).

Early postnatal exposure to PCB (8μg/g, 16μg/g, 32μg/g, 64 μg/g) in Holtzman rats was associated with decreased sperm ability to fertilize eggs but no significant differences in production, morphology or motility of the sperm. (29) A significant difference was found in the swimming path (reduced linearity or straightness) of the sperm in the 32 μg/g exposed group, but not in other groups so its significance is not clear.

A study done on male mice exposed to tetracholorobiphenyl pre and postnatally showed a significant difference in their fertilizing ability at 19 weeks of age but not at 9 weeks of age. (30) However, there were no differences in sperm concentration, velocity, linearity, or amplitude of lateral head displacement.

A similar study examined the effects of PCB, Aroclor 1242 (0, 10, 25, 50, 100 mg/kg-bw) was administered on mice and their offspring were studied. (31) This study found that the sperm count, velocity and linearity increased (near to significance), however the sperm fertilizing ability was significantly decreased in all PCB groups except the 50mg exposure group.

Exposure to DDT and fertility in mice

Studies done on rats exposed to DDT have shown similar results. Juvenile male rats (Wistar/Han strain) when fed on DDT (500mg/kg at 4^{th} and 5^{th} day or 200mg/kg daily from 4^{th} till 23^{rd} day of life) showed an increased physiological degeneration of the spermatogenetic cells and leydig cells. (32) Also the litters of these males were reduced in size significantly when compared to controls.

Wistar male rats (2-3months old) were administered DDT (50 and 100mg/kg body-weight) for 10 days and were found to have significantly reduced seminal vesicle weight 10 days after stopping administration. (33) The semen analysis showed significantly decreased spermatozoa, motility and a marked alteration of spermatogenic process.

The overall evidence for the association between PCB and DDE and their effects on male fecundability in animals is not consistent. Of the seven studies (1 in rhesus monkeys, 2 in mice and 4 in rats) four have shown that exposure to PCB was associated with a decline in fecundity, and three of them did not find a significant association. Human studies suggest that a negative association exists between PCB exposure and fecundity; however, these studies were not multi - generational except one.

Hypothesis and objective of the present study

The presence of high levels of PCBs and DDE in the Great Lakes has lead to three cross-sectional surveys. These were conducted between 1973-1991 among licensed sport fish anglers. They were selected on the basis of their consumption of fish. In 2000, we contacted the participants and collected information about their offspring. (34) The offspring were then recruited to participate in a study on their reproductive health. The present study focuses on the male offspring and the association between *in utero* PCB/DDE exposure and their fecundability. We hypothesize that *in utero* exposure to PCBs and DDE decreases fecundability in the male offspring.

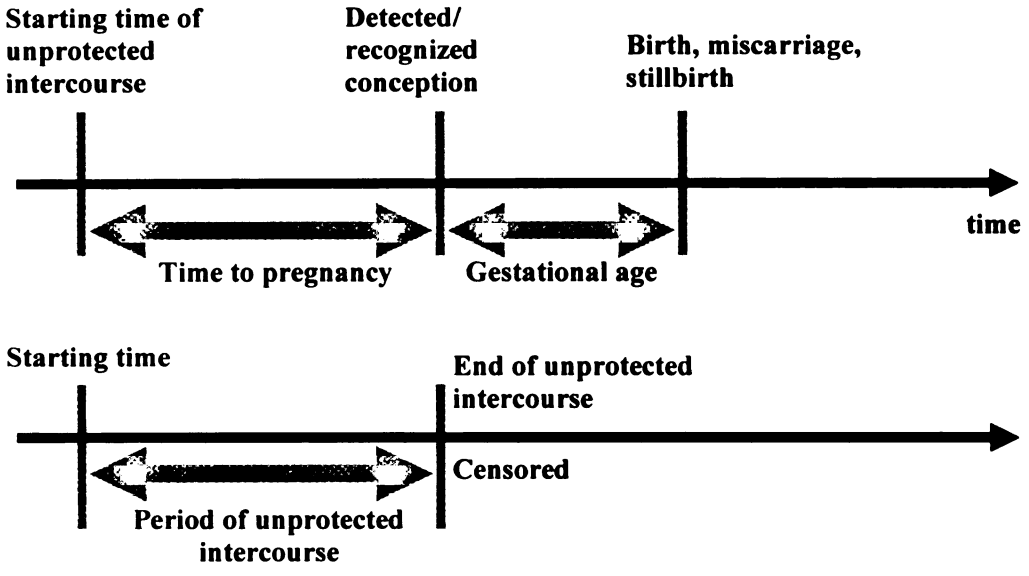
CHAPTER 2

Methodology

Definitions

The term fecundity is used for the biologic ability of conception. Fecundability characterizes the probability of conception in a population. When described in association with pregnancies fecundability has been described by WHO as "The monthly probability of conception in the absence of contraception outside the gestation period and the temporary sterile period following the termination of a pregnancy". (35) To evaluate fecundability it is important to determine the starting date of unprotected intercourse, the date of conception of a recognized pregnancy and the time period between these events. The term time of unprotected intercourse (TUI) is used for any time interval, which ended in a pregnancy, or not. The time intervals, which ended in a pregnancy, are well defined and the term time to pregnancy (TTP) is used to describe it. For those time intervals, which have not or not yet, lead to a pregnancy the term periods of unprotected intercourse (PUNP) is used. TUI comprises of both TTP and PUNP. (Figure 3)

Figure 3. Time to pregnancy and period of unprotected intercourse



Population

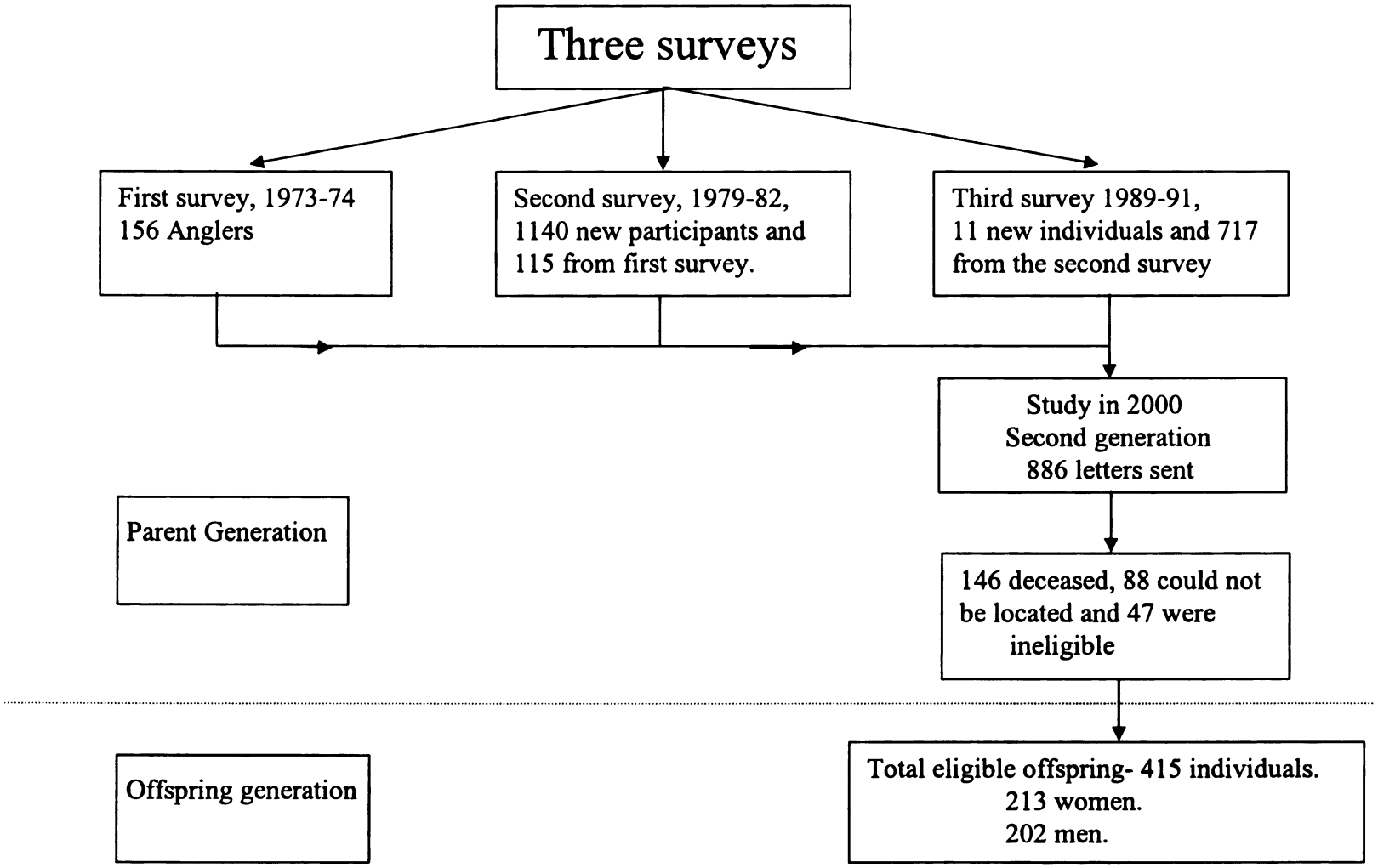
Background Information

Michigan Department of Community Health has conducted three cross-sectional surveys between 1973-1991, at fishing sites along the whole shore line of Lake Michigan. The target population was sport fish anglers. In the first survey in 1973-74, 156 anglers were recruited whose serum PCB levels were determined. In the subsequent survey in 1979 -82, there were 1,140 new participants and 115 from the previous one with blood measurements of DDE and PCB. The third survey in 1989-91 had 11 new participants in addition to the 717 participants from the second survey. The above surveys provided a total of 1,177 individuals whose PCB and DDE levels were determined. In all the three surveys, in addition to determining the PCB and DDE values, the participants also answered a questionnaire dealing with their fisheating habits in the Great Lakes.

In 2000, we approached the parental cohort of fisheaters again by telephone. Deceased were excluded based on Michigan Vital Records data until the summer of 1999 (n=119). The Michigan Department of Community Health sent out 886 letters to families or individuals representing 621 original families. If the couple was separated/ divorced a letter was sent to each individual. Approximately two weeks later, trained telephone interviewers called the participants and re-explained the purpose of the study. After a verbal consent was obtained, an interview was conducted regarding their offspring's birth characteristics. We could not locate 88 individuals; 27 were deceased after the summer of 1999 and 47 were ineligible as they had no children. Totally, we had 412 participant families with 647 individual parents with 1,050 offspring. We then restricted the study to male offspring who were between 20-50 years of age and whose maternal serum PCB and DDE concentrations were determined between 1973-1991. There were 202 eligible male offspring. (Figure 4)

The review board on human subjects of Michigan State University approved the study on the adult offspring. We then contacted the offspring by telephone, and sent consent forms to those who were willing to participate. After receiving signed consent forms we scheduled and conducted interviews with the offspring.

Figure 4. Fisheater cohort from three surveys (1973-1991) and their offspring



Data Collection

Questionnaire

The interview was conducted on telephone by trained male interviewers. The questionnaire comprised of general background questions - date of birth, race, marital status, occupation, height, weight, if the participant knew if his mother consumed sport caught fish, and if he was breast fed as a baby. We then asked them about their general health and reproductive health. The participant was asked if he has ever been diagnosed with prostatitis, epididymitis, inflammation or infection of the testicles, undescended testicles, testicular tumors, testicular torsion or twist, testicular trauma, biopsy, varicocele, inguinal hernia repair, a known low sperm count, erectile dysfunction, hypospadias, urinary tract infection and the age at the time of diagnosis of any of these conditions if present. We also inquired if they had fathered any pregnancies and if so, the number.

If the participant ever fathered a pregnancy, we continued questions about the time it took his partner/ wife to get pregnant, the duration of pregnancy, if they were planning for a baby at the time of that pregnancy and if they were using any birth control at that time. If the pregnancy occurred as a result of birth control failure we asked the participant about the form of birth control they used (i.e. oral contraceptives, intrauterine device, diaphragm, cervical cap, foam or jelly, sponge, rhythm method, checking cervical mucus, norplant, depo provera, partner had tubal ligation, had vasectomy, condoms). The time of unprotected intercourse before each pregnancy was recorded in weeks/ months/ years.

We also obtained information on the frequency of sexual intercourse during this time interval. For each time interval, information regarding the lifestyle of the male offspring and his partner/ wife (i.e. smoking cigarettes, alcohol and caffeine intake) was obtained. The participant was also inquired if he or his wife/ partner ever had any reproductive organ surgery, or any other conditions, which has made it difficult or impossible to father a child. Information regarding time intervals of more than two months of unprotected intercourse and the partner/wife still did not become pregnant was also asked. The interview lasted about 40- 60 minutes depending on the number of pregnancies each male offspring fathered.

Determination of PCB and DDE

In each of the three surveys conducted between 1973-1991, participants in the parent generation were requested to provide non-fasting blood samples for analyses of serum PCBs and other organochlorines. All serum samples were analyzed at the Health Risk Assessment Laboratory of the Michigan Department of Community Health. Serum PCB and DDE levels were determined, using a modification of the Association of the Official Analytical Chemists, approved Webb-McCall packed column gas chromatography technique. The laboratory analytical methods used are described in detail elsewhere. (36, 37) This procedure used methanol-ether /hexane extraction, microflorisil column cleanup, and silicon gel column separation technique before injection into a computer programmed electron detection chromatograph. In the 1973 - 1974 and 1979 - 1982 surveys, PCB determination was based on Aroclor 1254 and 1260 standards. In the 1989 -1991 survey, Aroclor 1016 and 1260 standards were used. In order to compare the exposures, we used PCB measurements based on the Aroclor 1260

standard, which were available for all samples. Laboratory values reported as less than the detectable limit for Aroclor 1260 (3 μg/kg) were assigned as 1.5μg/kg. Less than 5% of the samples were below the threshold in all three surveys. The detection limit for DDE was 1 μg/kg. DDE was not determined in the first survey (1973-1974).

Determination of PCB and DDE exposure at the time of birth.

Female participants in the fisheater cohort had one to three PCB and DDE determinations conducted in 1973–1974, 1979–1982, and 1989–1991. These maternal values do not directly reflect the organochlorine concentrations to which their offspring were exposed during pregnancy. The offspring were born between 1950-1980. In order to determine their *in utero* exposure we backward-extrapolated maternal concentrations. We tested three options (38)

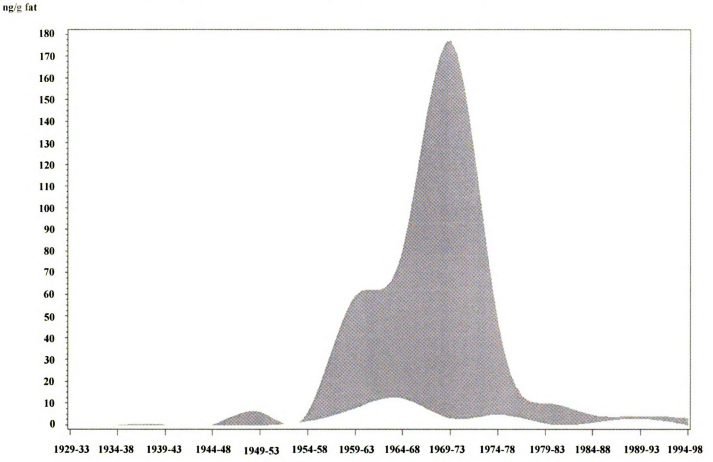
(1) A simple model based only on decay (39)

(2) A complex model incorporating decay, maternal body burden reduction due to pregnancy and breastfeeding, and a decline of exposure from fish due to a decrease in content of PCB and DDE (40)

(3) Linear regression models based on repeated measurements in our samples.

Additionally, we gathered information on PCB and DDE exposure in fish from Lake Michigan. Maximum and minimum fish concentration of PCB (and also DDE, data not provided), grouped into five-year periods, show an increase until 1969-1973 and a decrease thereafter (Figure 5). Hence, in this fisheating cohort we had to deal with persistent toxicants characterized by a high exposure in the past and a lower exposure after 1980.

Figure 5. PCB concentration* in fish from Lake Michigan, 1929-1998



* The upper and lower boundaries represent the minimum and maximum PCB concentrations detected in fish samples between 1929 – 1998.

Having repeated measurements (1973–1974, 1979–1982, and 1989 –1991) for female participants of the parent generation, we tested how well the three models outlined above could predict actual serum organochlorine measurements collected in the past. By means of intraclass correlation coefficients (ICC) (41) we compared estimated and measured past exposures. Briefly, the results showed that the simple model overestimated past exposure, as it did not take the lower PCB concentrations in the 1950s and 1960s into account. The complex model underestimated past exposure, as the estimated intake after 1980 lead to very low estimate than the actually measured exposure in the preceding years. However, with linear regression models, we identified two formulas that predicted past values with high precision (ICC = 0.77 and ICC=0.89, Table 1).

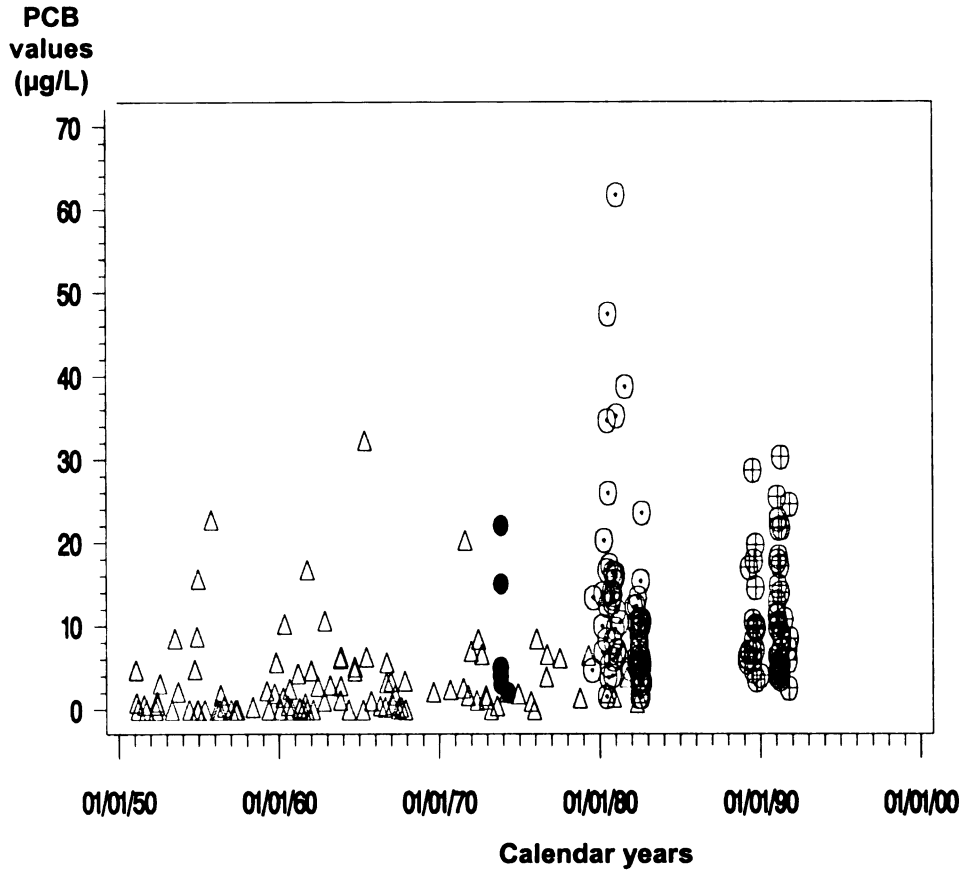
The first formula used the PCB value determined in the 3rd survey (1989–1991), the years that passed between the 2nd and 3rd determination, and the number of deliveries in between. Noteworthy, the years passed have a positive value indicating that the predicted value was higher before the 3rd measurement. The second formula gained a high prediction based on the PCB value of the 2nd determination, the years between the 2nd and 1st determination, and the years of fish consumption. This formula has a negative sign for the years passed between the two determinations, which indicate lower PCB values before 1979–1982. Therefore the estimations mirror the trends that were detected for PCB concentration (Figure 6).

Table 1. Backward extrapolation of maternal PCB and DDE serum concentrations

Extrapolation period	Estimated formula	Intraclass correlation coefficient
Extrapolation used between 1990 to 1980	PCB = 10**(-.1932438126 + log10 (PCB 3rd survey * 0.7807472978) + years between * 0.0488112342 + number of preceding births * -.1446091487	0.77 lower 5% limit: 0.71 (n=159)
Extrapolation used before 1980	PCB = PCB in the 2nd survey * 0.5647961666 + years between * -0.1633435551 + years of preceding fish consumption * 0.1059403879	0.89 lower 5% limit: 0.80 (n=22)

We applied these formulas to estimate the maternal serum PCB and DDE concentration at the time of the pregnancy. The circles in figure 5 represent the PCB measurements in the three period and the triangles the estimated values. The distribution of the estimated maternal PCB serum values mirrors the trend in PCB concentration in fish (Figure 6) with a delay of approximately 10 years.

Figure 6. PCB* serum levels determined in three surveys between 1973-1991 and their estimated values.



The ●, ⊙, ⊕ represent actual measurements and △ represents the estimated values.

Statistical Analysis

Fecundability is defined as the probability of conceiving. We assessed the probability of fathering a pregnancy in male offspring exposed to DDE and PCB *in utero*. Both TTP and PUNP contribute to TUI. TTP distributions cannot be directly translated into conception probabilities, as TUIs not or not yet resulting in a pregnancy (PUNP) are not included. Pregnancies, which ended as a result of birth control failure, were censored, as their time to pregnancy cannot be determined. Conception

probabilities and related estimates can be calculated when both pieces of information are analyzed together.

TUIs were calculated in three methods:

1. The information about the duration of TTPs and PUNPs provided by the participants.
2. TTPs were calculated using the date (month) when the wife/ partner of the participant conceived and subtracting from it the starting date of the period of unprotected intercourse.
3. Calculating TTP from the dates provided by the participant i.e. subtracting gestational age from the day the pregnancy ended and then calculating the difference to the date/month when they started trying to conceive.
4. In case of periods of unprotected intercourse not or not yet leading to pregnancy, the duration was calculated by subtraction of the starting date from the date the period ended, if ongoing, the interview date.

For TTPs we compared the durations derived from the three methods (1 - 3) described above. For PUNPs, we used two methods (1 and 4). In case of disagreements of more than a month, we reviewed all reproductive dates and corrected some values when two methods resulted in similar durations and the third was erroneous. In case of no agreement, we called the men again to clarify the dates and times. Additionally, we defined the age of the participants when having their first period of unprotected intercourse. Information in men who never attempted to conceive was censored.

We classified the male offspring exposure based on the extrapolated DDE and PCB concentrations into: DDE: 0 -<5μg/L (low), 5-< 7.5μg /L (medium), ≥ 7.5 μg /L (high); PCB: 0-<1 μg /L (low), 1-< 5 μg/L (medium), ≥5 μg /L (high). The following

confounders were controlled in the statistical analysis: Caffeine consumption/day was grouped into 0-<100 mg, 100- <300 mg, 300-<500 mg based on the participant's consumption of tea, coffee, iced tea and caffeinated beverages (50 mg of caffeine / cup of tea, 40 mg of caffeine / drink of caffeinated beverage, and 115mg of caffeine / cup of coffee). (42) We also classified the consumption of alcohol (beer, wine, liquor, cocktails) into three groups – no alcohol consumption, 0-0.5 drinks/day, and 0.5-30 drinks /day. We assumed that each of these drinks (12 oz. of beer, 6 oz. of wine, 1 oz. of liquor, mixed drinks and cocktails) provide an equal amount of alcohol (approximately 12 g). (43, 44)

Infections of reproductive organs (prostatitis, epididymitis, infections or inflammation of the testicles), testicular tumors, testicular torsion or twist, testicular trauma, biopsy, varicocele, inguinal hernia repair, a known low sperm count, erectile dysfunction, hypospadias and urinary tract infection were defined as risk factors whenever these disorders occurred before the starting date of the respective TUI.

Age of the participants at the starting time of each TUI in the study was grouped into 14-<20 years, 20-<25 years, 25-<30 years, and 30 years and above. The participants were classified by their birth dates into birth cohorts by the order 1950-1952, 1953-1962, 1963-1972, and 1973-1982. The age of the participant's partner was also grouped into 14- <20 years, 20 - <25 years, 25-<30 years, and 30 years and above.

Smoking status of the participant at the beginning of each TUI was categorized into no smoking, 0-10 cigarettes/day, >10 cigarettes/day. If the participant was breast fed as a baby and planning to have a baby at the beginning of TUI were collected as yes vs. no. For frequency of sexual intercourse at the beginning of TUI we asked for the actual

number per day, week, or month which we grouped into 0-7, >7-14, >14-28, and >28 times/ month.

For the combined analysis of TUIs we applied the Cox proportional hazards model using the 'proportional hazards regression model' (PHREG). (45, 46) TUIs without conception were treated as censored data. We estimated the fecundability ratio (FR, hazard ratio) and its 95% confidence interval. To account for the occurrence of ties, exact maximum likelihood estimates were calculated. The PHREG procedure also provides survival probabilities for not conceiving and the median time to conception. We adjusted the effect of *in utero* DDE and PCB exposure on the fecundability ratio (FR) for the offspring breastfed as a baby, any genital tract infection/before TUI, smoking, alcohol, and caffeine consumption, planning a baby and the frequency of sexual intercourse during TUI, and by stratification for age at the beginning of the TUI, education, and birth cohorts.

For descriptive purposes, we provide information on the male offspring. Units of analysis in this study, however, are the TUIs. As one man could contribute more than one TUI, observations are correlated. To investigate, if the association changed when only one TUI from each male offspring was used; we also estimated the FR based on last TUI. Lastly, we analyzed a model that included only TTP, excluding all periods that did not result in a pregnancy.

CHAPTER 3

Results

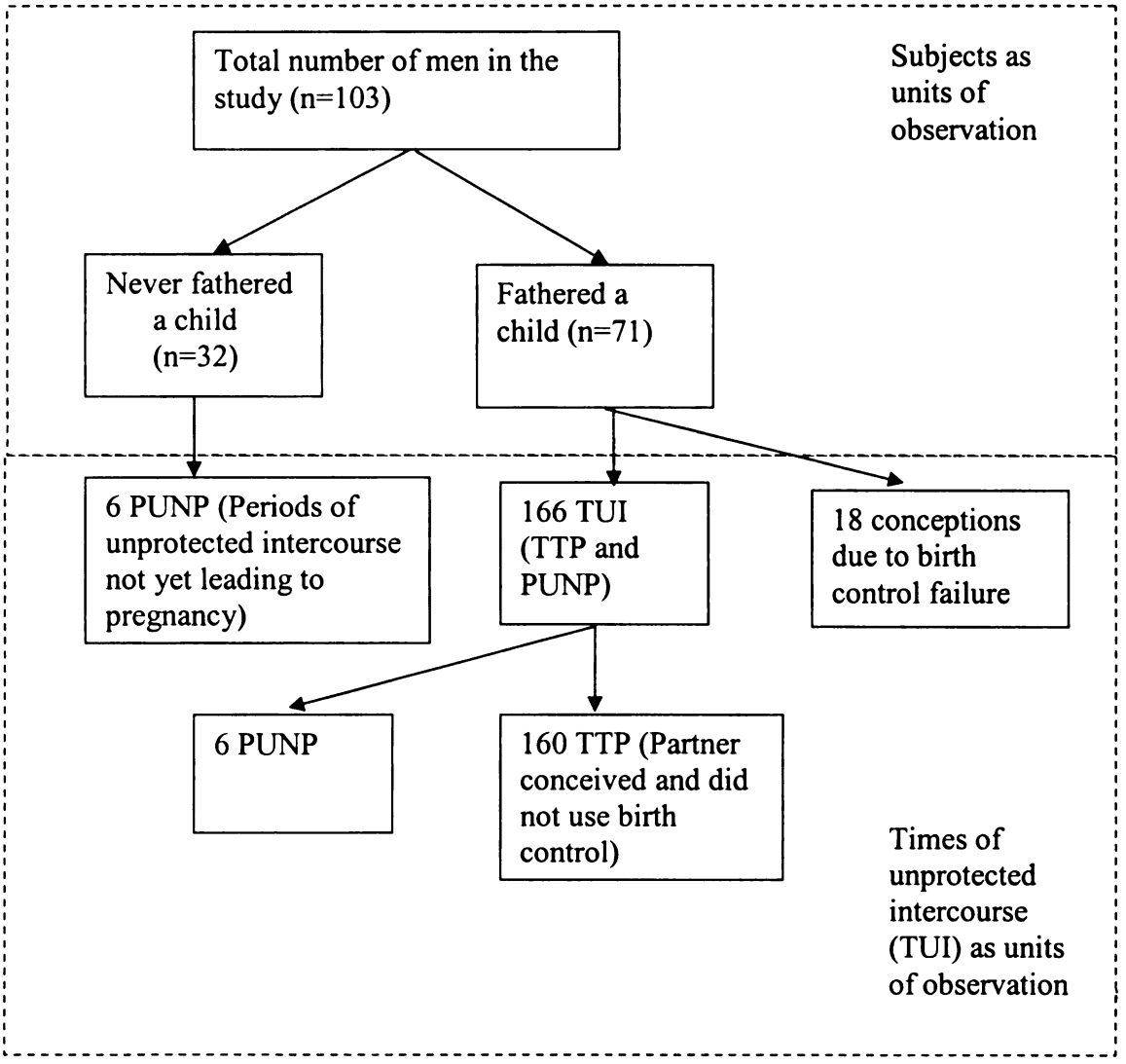
We identified 202 eligible male offspring of which 103 (50.9%) participated. Of the 103 men 71 have ever fathered a pregnancy and 32 did not. In those (n=71) who fathered a pregnancy the majority were between 30- 50 years, were married, and were college graduates. The men who never fathered a pregnancy were younger, in the 20-30 year age group and single. (Table 2) The age when the participants first attempted to father a pregnancy was not different between the ever and the never fathered men. Three of the participants who ever fathered a pregnancy were unable to provide their age at beginning of their TUI as these pregnancies resulted from birth control failure. Breastfeeding status as an infant among the male offspring was significantly different between those who fathered a child and those who did not with a majority of who never fathered a child being breastfed. Fertility problems were also higher among the participant's partners' who had a child (Table 2).

Table 2. Description of the male offspring (%) of the Fisheater Family Health study, 2000-2001

Characteristics		Men who ever fathered a child (n=71)	Men who never fathered a child (n=32)
Birth cohort:	1943 to 1952	11.3	3.1
	1953 to 1962	52.1	12.5
	1963 to 1972	31.0	40.6
	1973 to 1982	5.6	43.8
Age at the time of the interview	20 - <30yrs	8.5	56.3
	30 -<40 yrs	35.2	28.1
	40 -<50 yrs	50.7	15.6
	≥ 50 yrs	5.6	0.0
Education	High school graduate	9.9	6.3
	Associate degree	23.9	56.3
	College graduate	28.2	21.9
	Graduate school	38.0	15.6
Marital status	Married	94.4	31.3
	Single but living together	1.4	9.4
	Single	2.8	56.3
	Others (engaged, divorced)	1.4	3.1
Mother ate sport caught fish (information from the offspring)	Yes	49.3	28.1
	No	21.1	43.8
	Don't know	29.6	28.1
Breastfed as an infant	Yes	57.8	81.3
	No	29.6	9.4
	Don't know	12.7	9.4
Ever had fertility problems (offspring)		14.3	9.4
Partner had infertility problems		18.8	8.3
In utero DDE exposure (serum concentration in the mother in µg/L)	< 5	54.8	65.5
	5 - < 7.5	19.4	13.8
	≥ 7.5	25.8	20.7
In utero PCB exposure (serum concentration in the mother in µg/L)	<1	45.1	31.3
	1 - <5	33.8	43.8
	≥ 5	21.1	25.0
Age at the time of first TUI (time of unprotected intercourse) (years)	14 - <20	10.1	0.0
	20 - <25	14.5	16.7
	25 -< 30	40.6	50.0
	≥ 30	34.8	33.3

Participants provided 190 times of unprotected intercourse (TUI), of which 18 cannot be defined as TUI as they were using birth control. (Figure 7) Of the remaining 172 TUI, 160 ended in a pregnancy (TTP), while 6 TUI did not end in a pregnancy (PUNP) in the men who ever fathered a child. The men who never fathered a child contributed 6 time periods of unprotected intercourse not yet ending in a pregnancy (PUNP). Finally we analyzed 172 TUI (160 TUI + 6 PUNP + 6 PUNP).

Figure 7. The distribution of TUI (times of unprotected intercourse) among the male offspring, in the Fisheater Family Health Study, 2000 - 2001.



TUI (Table 3) and saw that men in the 1963-1972 birth cohort had a longer TUI (3 months) when compared to those in the other birth cohorts. Men who were < 20 years old at the beginning of TUI had a longer TUI (4 months) when compared to the men in the other age groups. Men who were smoking and whose female partner was smoking had a longer TUI (3 months) when compared to their nonsmoking counterparts (2 months). No alcohol consumption and lower sexual frequency were also associated with a longer TUI (Table 3).

Table 3. Median TUI (Times of unprotected intercourse) in relation to various exposure variables, which may influence TUI in the Fisheater Family Health study, 2000-2001.

Off spring characteristics (Participant)		Median TUI (months)	P5 - P95
Birth cohort	1943 to 1952	1.5	0.23 – 84.0
	1953 to 1962	2.0	0.23- 32.0
	1963 to 1972	3.0	0.47-30.0
	1973 to 1982	2.0	0.23-7.0
Age at the beginning of TUI (yrs)	14 < 20	4.0	2.0-25.0
	20 -< 25	2.0	0.35-32.0
	25 -< 30	2.0	0.23-15.0
	30 and above	2.0	0.23-27.0
Breastfed as an infant	Yes	2.0	0.23-32.0
	No	2.0	0.47-30.0
Participant smoking	Yes	3.0	0.69-32.0
	No	2.0	0.23-30.0
Participant's female partner smoking	Yes	3.0	1.0-24.0
	No	2.0	0.23-32.0
Alcohol consumption (g/ day)	No	16.0	0.47-84.0
	≤ 0.5	2.0	0.23-25.5
	> 0.5	2.5	0.47-28.5
Caffeine consumption (mg / day)	≤ 100	2.0	0.47-30.0
	101-300	3.0	0.47-32.0
	> 300	2.5	0.23-32.0
Sexual frequency (times / month)	0 - ≤ 7	6.0	1.0-37.0
	>7 - ≤ 14	2.0	0.23-24.0
	>14 - ≤ 28	2.0	0.47-25.0
	> 28	1.69	0.61-17.2

Male offspring who were breastfed as infants and those offspring who received graduate and higher education were exposed to higher PCB (> 5 μg/L) and DDE (>7.5 μg/L) levels *in utero*. Offspring exposed to medium PCB (1-<5μg/L) and DDE (5-<7.5 μg/L) *in utero* were associated with significantly higher cigarette smoking. Caffeine intake was also significantly higher in the offspring exposed to medium PCB but not in association with DDE (Table 4).

Age at the beginning of TUI, alcohol intake, sexual frequency during TUI, was not significantly associated with exposure to PCB and DDE. (Table 4) However, the age at the time of first TUI in the medium PCB exposure group was lower (26.6 years) when compared to the low PCB (27.4 years) and high PCB (28.7 years) levels of exposure. When the starting age was compared to the DDE exposure there was a gradual increase in the age with increased exposure (26.5, 26.1, 28.5 years). For this analysis men who were never at risk of conception were censored and age at interview was used instead. Controlling for the education of the offspring, birth year, and breastfeeding we did not find a significant increase in age at attempt in the exposure groups.

Table 4. Description of the variables (%) determined at the beginning of TUI, in the Fisheater Family Health Study, 2000- 2001

		TUI (n=172)	*In utero* PCB exposure (µg/L)			*In utero* DDE exposure (µg/L)		
			< 1 (n=74)	1 < 5 (n=56)	≥ 5 (n=42)	<5 (n=82)	5 - < 7.5 (n=26)	≥ 7.5 (n=40)
Age at TUI (years)	14 - <20	4.1	2.7	5.4	4.8	6.1	3.9	2.5
	20 - <25	12.2	13.5	14.3	7.1	18.3	3.9	12.5
	25- <30	31.4	25.7	37.5	33.3	29.3	42.3	30.0
	>30	52.3	58.1	42.9	54.8	46.3	50.0	55.0
Offspring smoking during TUI		23.8	29.7	32.1	2.4†	24.4	53.9	5.0†
Offspring's partner smoking during TUI		7.6	6.8	12.5	2.4	8.5	7.7	0.0
Caffeine Intake/day	0-100 mg	25.3	23.3	28.9	24.4	21.9	47.6	30.8
	100-300 mg	31.3	31.5	44.2	14.6 †	34.2	23.8	23.1
	>300 mg	43.4	45.2	26.9	61.0	43.9	28.6	46.2
Alcohol intake/day (drinks)	No	7.0	9.5	7.1	2.4	8.5	0.0	2.5
	0 - 0.5	34.9	40.5	32.1	28.6	46.3	19.2	25.0 †
	>0.5	58.1	50.0	60.7	69.1	45.1	80.8	72.5
Genital tract disease before TUI	Yes	19.8	14.9	23.2	23.8	18.3	3.9	27.5
Education of the offspring	High school	9.3	16.2	7.1	0.0 †	12.2	0.0	15.0 †
	Associate degree	29.1	21.6	42.9	23.8	41.5	34.6	5.0
	Graduate and more	61.6	62.2	50.0	76.2	46.3	65.4	80.0
Sexual frequency (times/month)	0 < 7	14.6	14.1	19.6	9.8	9.5	20.8	21.1
	7 <14	43.0	36.6	47.8	48.8	46.0	50.0	42.1
	14 ≤ 28	29.8	35.2	23.9	26.8	29.7	25.0	31.6
	> 28	12.7	14.1	8.7	14.6	14.9	4.2	5.3
Planning a baby		83.5	83.6	81.8	85.7	79.3	84.6	82.5
Breastfed as an infant		47.7	36.	50.0	64.3†	42.5	23.1	65.0†

† - Significantly different in the exposure groups

To analyze fecundability in the offspring we controlled for the covariates – birth cohort, age at the starting time of TUI and education obtained by the offspring in the survival analyses, we had a sample of 155 TUI with information missing on breastfeeding (n=2), sexual frequency (n=14). Medium PCB exposure was associated with higher fecundability ratio (2.7), which was statistically significant. With DDE, higher exposure was associated with a higher FR (1.95), however they were not significant. Medium alcohol consumption (0-<0.5 g /day) was associated with a high FR of 10.82 and high alcohol consumption (>0.5g/day) was associated with a FR of 6.18. Frequency of sexual intercourse showed a positive association with FR (Table 5).

However, when the analysis was restricted to those who consumed alcohol there was a sample size of 143 TUI. There was no significant increase in the FR (2.97, 95% CI – 1.46 – 6.05) and high level of PCB exposure (FR=0.93, 95% CI- 0.45 – 1.91).

Table 5. Adjusted Fecundability† Ratios in relation to various variables that may influence TUI (Time of unprotected intercourse) in the Fisheater Family Health study, 2000-2001.

Variable		Total (n=155) Fecundability ratio (FR)	95% CI
PCB exposure *in utero* (μg/L)	1-<5	2.7	1.35-5.39
PCB exposure *in utero* (μg/L)	>5	0.91	0.45-1.83
DDE exposure *in utero* (μg/L)	5-<7.5	1.35	0.65 -2.80
DDE exposure *in utero* (μg/L)	>7.5	1.95	0.97-3.91
Offspring was breastfed as a child		1.55	0.88-2.72
Genital tract disease before the TUI		0.87	0.46-1.68
Smoking at the beginning of TUI		0.88	0.33-2.37
Partner smoking at the beginning of TUI		2.15	0.78-5.96
Alcohol consumption at the beginning of TUI (drinks)	0	1	
	0-<0.5	10.82	2.22-52.77
	0.5 -<30	6.18	1.38-27.72
Caffeine consumption at the beginning of TUI (mg/day)	0-<100	1	
	100-<300	0.82	0.41-1.62
	300-<500	1.10	0.60-2.03
Frequency of sexual intercourse	0-7 times/month	1.0	
	7-14 times/month	1.80	0.79-4.07
	14-28 times/month	2.0	0.82-4.85
	>28 times /month	4.3	1.48-12.48
Planning a baby		4.42	1.86-10.48

† = Adjusting for birth cohort (1950-1952, 1953-1962, 1963-1972 and 1973-1982), age at the starting time of TUI (14-<20 yrs, 20- <25 yrs, 25- <30yrs and ≥ 30yrs) and education of the male offspring of the Fisheater Family Health Study, 2000-2001.

Planning a baby was associated with a significantly high FR (4.42). When the analysis was restricted to those who planned a baby there were 129 TUI with FR in the medium PCB exposure group showing a slight increase (FR = 2.9, 95% CI= 1.28- 6.47) with no change in the high PCB exposure group.

When the analysis was restricted to the last TUI from each offspring we had 63 TUI, with FR = 0.55 in the medium PCB exposure group (95% CI= 0.08 – 3.99) and FR=4.05 (95% CI = 0.36-45.15) in the high PCB exposure group. The FR of DDE exposure changed significantly with FR= 22.17 (95% CI= 1.27-386.35) in the medium DDE exposure group and FR = 1.13 (95% CI = 0.15-8.8) in the high DDE exposure group.

CHAPTER 4

Discussion

This study investigated the influence of *in utero* PCB and DDE exposure on male offspring fecundability. Our findings suggest that medium PCB exposure (1-< 5 μg/L) was associated with higher fecundability when compared to the low (< 1 μg / L) and high

(≥ 5 μg / L) levels of PCB exposure, which is contradictory to our hypothesis. Additionally, we could not reject the null hypothesis for DDE.

Of the 202 eligible male offspring of the Michigan fisheater cohort, 103 (50.7%) agreed to participate, 37 declined to participate (18.3%) and we could not contact the rest due to change of address or phone numbers. The sample size is small (n=103) and is limited as this is a second generation study. The small sample may have lead us to miss weak associations between PCB and DDE and related changes in fecundability.

One of the main strengths of our study is the availability of maternal serum PCB and DDE exposures during three past investigations. The information collected from the questionnaires during these three surveys helped us in estimating the *in utero* PCB and DDE exposures at the time of birth of each offspring. This helped us in getting a reliable estimate of the *in utero* PCB and DDE exposure.

One of the limitations we have with PCB exposure is lack of information about individual congeners. We used Aroclor 1260 as PCB exposure. This may have a problem as various PCB congeners have different endocrine effects, (47) which may have modified the association between PCB and fecundity. Also, we do not have information

on the PCB, DDE levels in the offspring and their partners.

Reproductive epidemiology studies are vulnerable to many biases as described by Weinberg. (48) The subjects had to recall events in their reproductive history, which can lead to an information bias. When scheduling an interview, the interviewers requested the participant to have their wife/ partner around so that she could help the participant regarding the TUI. However, this was not possible in all the interviews. Further, to avoid any inconsistencies in the time intervals for TUI we asked the participants for dates and also for time durations so that we can investigate any inconsistencies and correct them. The median recall duration for the first attempt to conceive was 12.3 years. Joffe showed that TUIs could be recalled with high specificity and sensitivity even over longer recall periods. (49) However, lack of precise recall may have introduced a non-differential misclassification.

Behavior modification bias can result when men who had long TUI may have had a change in their behavior (smoking, alcohol consumption), which they may have perceived as unhealthy. The information collected in this study is at the beginning of TUI. Hence we attempted to diminish this bias.

Use of birth control may not be consistent in the couples and a planning bias would result if couples in the various exposure groups have different rates of birth control failure. We had 18 pregnancies as result of birth control failure (18/ 294 = 6.1%). Of all the birth control failures the highest proportion was in the low DDE (78.6%) and low PCB (55.6%) exposure group.

Couples, who were less careful about using birth control, conceive and may be more fertile, which may introduce a bias. In our study the number of pregnancies as a

result of birth control failure comprised 6.1% (n=18), of which 10 were present in low PCB exposure group, seven in the medium PCB exposure group, and one in the high PCB exposure. In association with DDE exposure, 11 pregnancies due to birth control failure were in the low DDE exposure group, 2 in the medium DDE exposure, one in the high DDE exposure with the DDE exposure missing in four of them. The presence of a high proportion of birth control failures in the low PCB and DDE (78.6%, 55.6%) exposure groups maybe an indirect indicator that they are more fertile which may introduce a bias as these are excluded from the analysis. However, the proportion of pregnancies due to birth control failure is small (6.1%), which may have not had a significant influence on the total sample.

Another bias could result if the couple is at risk of conceiving prior to they completely go off the birth control especially if they are using barrier methods. In order to overcome this bias, Weinberg (48) suggested that all TUI which are of <1 month duration to be excluded from the analysis. The FR did not change substantially except in high PCB exposure group (FR=1.53, p=0.43 compared to FR=0.91, p=0.8) which was not significant. These findings suggest that there is no wantedness bias in our sample.

Pregnancy recognition bias can result because a pregnancy not yet recognized may undergo spontaneous abortion and may be misconstrued as a regular menstrual cycle. In order to avoid this, the occurrence of spontaneous abortions (21/172 = 13.1%) in various PCB and DDE exposure groups was analyzed. Low PCB exposure (< 1 μg/L) was associated with eight (38.1%), medium PCB exposure (1 <5 μg/L) with five (23.8%) and high PCB exposure (> 5 μg/L) with eight (38.1%) spontaneous abortions. Similarly, in association with low DDE exposure (<5 μg/L) there were nine (52.9%),

three (17.7%) in the medium DDE exposure (5 - < 7.5 μg/L) and five (29.4%) spontaneous abortions in the high DDE (> 7.5 μg/L) exposure group. There was no significant difference in the spontaneous abortions in the various levels of PCB (p=0.53) and DDE (p=0.94) exposure.

Medical intervention bias results when the couple took treatment to increase the chances of conception. In order to avoid this TUI ≥ 12 months were censored and the FR in the medium PCB exposure group changed to 2.51 (95% CI = 1.23 – 5.11) and in the high PCB exposure group FR = 1.03 (0.43 – 2.45).

In this study we investigated the influence of PCB and DDE on fecundity. These compounds are lipophilic, they co-exist in lipid tissues and are thus correlated ($r_{Spearman}$=0.55). In order to determine the combined influence of PCB and DDE on fecundity we combined the exposures i.e. the reference group had a low combined with a medium category, of either DDE and/or PCB; one group with both medium DDE and PCB categories, one group with high exposure to DDE and low levels of PCB; another with high levels of PCB and low levels of DDE, another with high levels of both PCB and DDE. With this approach of exposure classification the FR for medium DDE and PCB exposure was 4.9 (p=0.003) and for high DDE combined with medium/low PCB exposure 2.2 (p=0.24). Exposure to high PCB combined with medium / low DDE exposure lead to FR= 0.73 (p=0.58) and high DDE and PCB exposure with a FR= 1.4 (p=0.30). These findings are similar to our earlier findings in which we estimated fecundability in relation to PCB and DDE separately without combined effects. Their FR decreased with increasing exposure to PCB and increasing FR with increasing exposure to DDE with medium exposure to PCB and DDE being associated with a high FR

compared to other combined exposures.

DDE was associated with no decrease in fecundability with increasing exposure, which was contradictory to our hypothesis. The findings in our study are contradictory to the results from the other studies, which have shown DDE exposure is associated with a decline in fecundability as it as a potent androgen receptor antagonist. (50, 51)

The findings in our study that medium level of PCB exposure is associated with an increase in fecundability whereas low and medium levels of exposure are associated with a decline in fecundability may be associated with the combined estrogenic and anti-estrogenic action of the various PCB congeners. (52)

We found in our study that moderate level of alcohol consumption (< 0.5g/ day) was associated with a high fecundability when compared to no alcohol and high level of alcohol consumption. This may be in agreement with the findings in a study by Florack et al., which showed that moderate alcohol consumption was associated with a higher fecundability. (53)

To our knowledge this is the second study, to study the influence of maternal PCB and DDE on fecundability in the male offspring. Our findings do not suggest that fecundability may be influenced *in utero.* Also not all by animal studies found an adverse effect of PCB or DDE *inutero* exposure on fecundability. Nevertheless, further research studying the influence of organochlorine compounds on the fecundability of offspring is warranted.

REFERENCES

References

1. Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993; 101(5): 378-84.

2. Cocco PL. Environmental exposure to p,p'-DDE and human fertility. Bull Environ Contam Toxicol 1997; 59(5): 677-80.

3. Fisher BE. Most unwanted. Environ Health Perspect 1999;107(1):A18-23.

4. Waller DP, Presperin C, Drum ML, Negrusz A, Larsen AK, van der Ven H, et al. Great Lakes fish as a source of maternal and fetal exposure to chlorinated hydrocarbons. Toxicol Ind Health 1996; 12(3-4): 335-45.

5. Jacobson JL, Fein GG, Jacobson SW, Schwartz PM, Dowler JK. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. Am J Public Health 1984; 74(4):378-9.

6. McGrath M. The Fourth Coast. EPA Journal 1980; 6(5): 22-24.

7. International Joint Commission. Great Lakes water quality agreement of 1978. 1978.

8. International Joint Commission. Great Lakes water quality agreement of 1983. 1983.

9. International Joint Commission. A strategy for virtual elimination of persistent toxic substances. 1993; Volume I (Washington D.C, USA, and Ottawa, Ontario, Canada).

10. Frank M.D'ltri MAK. PCBs: Human and environmental hazards. 1983.

11. Matthews HB, Dedrick RL. Pharmacokinetics of PCBs. Annu Rev Pharmacol Toxicol 1984; 24:85-103.

12. Schecter A, Cramer P, Boggess K, Stanley J, Olson JR. Levels of dioxins, dibenzofurans, PCB and DDE congeners in pooled food samples collected in 1995 at supermarkets across the United States. Chemosphere 1997; 34(5-7): 1437-47.

13. WHO working group. DDT and is derivatives - environmental aspects. Environmental Health Criteria 1989; PG:98 p YR:1989 IP:83 VI

14. Schwartz PM, Jacobson SW, Fein G, Jacobson JL, Price HA. Lake Michigan fish consumption as a source of polychlorinated biphenyls in human cord serum, maternal serum, and milk. Am J Public Health 1983; 73(3): 293-6.
15. Humphrey HE, Budd ML. Michigan's fisheater cohorts: a prospective history of exposure. Toxicol Ind Health 1996; 12(3-4): 499-505.

16. Kostyniak PJ, Stinson C, Greizerstein HB, Vena J, Buck G, Mendola P. Relation of Lake Ontario fish consumption, lifetime lactation, and parity to breast milk polychlorobiphenyl and pesticide concentrations. Environ Res 1999; 80(2 Pt 2):S166-S174.

17. Slama R, Eustache F, Ducot B, Jensen TK, Jorgensen N, Horte A, et al. Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. Hum Reprod 2002; 17(2): 503-15.

18. Bonde JP, Hjollund NH, Kolstad HA, Abell A, Larsen SB. Environmental semen studies--is infertility increased by a decline in sperm count? Scand J Work Environ Health 1999; 25(Suppl 1): 12-6; discussion 76-8.

19. Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. Lancet 2000; 356(9237):1240-1.

20. Courval JMD, J.V. Stein, A.D Tay, E.M. He, J. Humphrey, H.E. Paneth, N. Sport-caught fish consumption and conception delay in licensed Michigan anglers. Environ Res 1999; 80(2 Pt 2): S183-S188.

21. Pines A, Cucos S, Ever-Handani P, Ron M. Some organochlorine insecticide and polychlorinated biphenyl blood residues in infertile males in the general Israeli population of the middle 1980's. Arch Environ Contam Toxicol 1987; 16(5): 587-97.

22. Bush B, Bennett AH, Snow JT. Polychlorobiphenyl congeners, p,p'-DDE, and sperm function in humans. Arch Environ Contam Toxicol 1986; 15(4): 333-41.

23. Dallinga JW, Moonen EJ, Dumoulin JC, Evers JL, Geraedts JP, Kleinjans JC. Decreased human semen quality and organochlorine compounds in blood. Hum Reprod 2002; 17(8): 1973-9.

24. Dhooge W, Stuyvaert S, Kaufman JM, Koppe G, Nele V, Schoeters G, et al. Observations as to male fertility in the Flemish environment and health studies. Folia Histochem Cytobiol 2001; 39 (Suppl 2): 38-9.

25. Hauser R, Altshul L, Chen Z, Ryan L, Overstreet J, Schiff I, et al. Environmental organochlorines and semen quality: results of a pilot study. Environ Health Perspect 2002; 110(3): 229-33.

26. Rozati R, Reddy PP, Reddanna P, Mujtaba R. Role of environmental estrogens in the deterioration of male factor fertility. Fertil Steril 2002;78(6):1187-94.

27. Allen JR, Barsotti DA, Lambrecht LK, Van Miller JP. Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann N Y Acad Sci 1979; 320: 419-25.

28. Linder RE, Gaines TB, Kimbrough RD. The effect of polychlorinated biphenyls on rat reproduction. Food Cosmet Toxicol 1974; 12(1): 63-77.

29. Sager DB, Shih-Schroeder W, Girard D. Effect of early postnatal exposure to polychlorinated biphenyls (PCBs) on fertility in male rats. Bull Environ Contam Toxicol 1987; 38(6): 946-53.

30. Huang A, Powell D, Chou K. Pre- and postnatal exposure to 3,3',4,4'-tetrachlorobiphenyl: I. Effects on breeding ability and sperm fertilizing ability in male mice. Arch Environ Contam Toxicol 1998; 34(2): 204-8.

31. Fielden MR, Halgren RG, Tashiro CH, Yeo BR, Chittim B, Chou K, et al. Effects of gestational and lactational exposure to Aroclor 1242 on sperm quality and in vitro fertility in early adult and middle-aged mice. Reprod Toxicol 2001; 15(3): 281-92.

32. Krause W, Hamm K, Weissmuller J. The effect of DDT on spermatogenesis of the juvenile rat. Bull Environ Contam Toxicol 1975; 14(2): 171-9.

33. Ben Rhouma K, Tebourbi O, Krichah R, Sakly M. Reproductive toxicity of DDT in adult male rats. Hum Exp Toxicol 2001; 20(8): 393-7.

34. Karmaus W, Huang S, Cameron L. Parental concentration of dichlorodiphenyl dichloroethene and polychlorinated biphenyls in Michigan fish eaters and sex ratio in offspring. J Occup Environ Med 2002; 44(1): 8-13.

35. WHO. Dictionary of Demographic and Reproductive Health Terminology. 1999.

36. Needham L, Burse V, Price H. Temperature-programmed gas chromatographic determination of polychlorinated and polybrominated biphenyls in serum. J Assoc Off Anal Chem 1981; 64(5): 1131-7.

37. Price HA, Welch RL, Scheel RH, Warren LA. Modified multiresidue method for chlordane, toxaphene, and polychlorinated biphenyls in fish. Bull Environ Contam Toxicol 1986; 37(1): 1-9.

38. Karmaus W, Fussman C, Muttineni J, Zhu X. Backward estimation of exposure to organochlorine using repeated measurements. Manuscript submitted 2003.

39. Weisskopf MG, Anderson HA, Hanrahan LP. Decreased sex ratio following

maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: a retrospective cohort study. Environmental Health 2003; 2(1).

40. Rylander L, Stromberg U, Dyremark E, Ostman C, Nilsson-Ehle P, Hagmar L. Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to low birth weight. Am J Epidemiol 1998; 147(5): 493-502.

41. Armstrong B, White E, Saracci R. Principles of Exposure Measurement in Epidemiology. Monographs in Epidemiology and Biostatistics. 1992.

42. Bolumar F, Olsen J, Boldsen J. Smoking reduces fecundity: a European multicenter study on infertility and subfecundity. The European Study Group on Infertility and Subfecundity. Am J Epidemiol 1996; 143(6): 578-87.

43. Hakim RB, Gray R.H, Zacur H. Alcohol and caffeine consumption and decreased fertility. Fertility sterility 1999; 70(4): 632-7.

44. Juhl M, Nyboe Andersen AM, Gronbaek M, Olsen J. Moderate alcohol consumption and waiting time to pregnancy. Hum Reprod 2001; 16(12): 2705-9.

45. Cox D. Regression models and lifetables. J R Stat soc series 1972;34:187-220.

46. SAS Institute. SAS/STAT Software. In. Cary, NC: SAS Institute Inc.; 2002.

47. Kodavanti PR, Kannan N, Yamashita N, Derr -Yellin EC, Ward TR, Burgin DE, et al. Differential effects of two lots of aroclor 1254: congener-specific analysis and neurochemical end points. Environmental Health Perspectives 2001; 109(11): 1153-61.

48. Weinberg CR, Baird DD, Wilcox AJ. Sources of bias in studies of time to pregnancy. Stat Med 1994; 13(5-7): 671-81.

49. Joffe M. Methods for obtaining valid data on time to pregnancy among men and women. Scand J Work Environ Health 1999; 25(Suppl 1): 8-9.

50. Akingbemi BT, Hardy MP. Oestrogenic and antiandrogenic chemicals in the environment: effects on male reproductive health. Ann Med 2001; 33(6):391-403

.

51. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature 1995; 375(6532): 581-5.

52. Jansen HT, Cooke PS, Porcelli J, Liu TC, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod Toxicol 1993; 7(3): 237-48.

53. Florack EI, Zielhuis GA, Rolland R. Cigarette smoking, alcohol consumption, and caffeine intake and fecundability. Prev Med 1994; 23(2): 175-80.