

## Review Article

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# The influence of perinatal and current dioxin and PCB exposure on reproductive parameters (sex-ratio, menstrual cycle characteristics, endometriosis, semen quality, and prematurity): a review

**Abstract:** Polychlorinated biphenyls (PCBs) and dioxins (PCDDs/Fs) are well-known endocrine disruptors. This paper strives to elucidate the data on reproductive consequences of perinatal dioxin and PCB exposure in men and women. We focused on the following end-points: sex-ratio, endometriosis, menstrual cycle characteristics, sperm quality, and prematurity. We summarize 46 papers and compare their results including effects seen after exposure to background concentrations. Seven of twelve studies showed a decrease in sex-ratio after parental dioxin or PCB exposure. In three of the seven studies, effects were seen after paternal exposure and in three after maternal exposure. In eight of the nine studies on menstrual cycle characteristics, abnormalities were associated with PCB or dioxin exposure, however the results differed. In three studies PCB and TCDD were associated with longer menstrual cycles, while three studies indicated that an increase in PCB/PCDF exposure was associated with shorter cycles. Five studies showed effects on menstrual bleeding with higher PCB or dioxin exposure. A higher

rate of irregular menstrual cycles in exposed women was seen in four studies. The conflicting outcomes probably result from variability in study design, timing of exposure and endocrine disrupting properties of the measured congeners. Nine of sixteen studies detected higher PCB or dioxin exposure in women with endometriosis. However, the manner of diagnosing endometriosis and the character of the studies varied from prospective to retrospective. Five of eight studies focusing on sperm quality showed that men, with higher serum concentrations of PCBs and/or PCB congeners and/or PCDFs, had reduced sperm quality, including increased abnormal morphology and reduced motility. The exposure timeframe seemed important here. There are two studies addressing preterm birth in relation to PCBs, one mentioned a shortening of three days of gestational age, two other studies did not find a relation. Recently one study related a shorter gestational age of half a week with overall dioxin activity measured with the CALUX method in cord blood, particularly in boys. In conclusion, exposure to PCBs and dioxins has a negative effect on the reproductive systems of human populations. Although some speculations have been made, the exact mechanism of these effects and the interactions of these compounds with other endocrine disruptors are not yet known. Age at exposure and congener specific properties are probably crucial in interpreting the observed results.

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## List of abbreviations:

CALUX	Chemical Activated Luciferase gene eXpression
DDT	Dichlorodiphenyl dichloroethene
DDE	Dichlorodiphenyl dichloroethylene
DLC	Dioxin like compounds
ER	Estrogen receptor
GC/MS	Gas Chromatography/ Mass Spectrometry; machine used for identification and quantification of (toxic) compounds
PBDE	Polybrominated diphenylether; brominated diphenylether
PBB	Polybrominated biphenyl
PCB	Polychlorinated biphenyl
dl-PCB	dioxinlike Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo- <i>p</i> -dioxin; dioxin
PCDF	Polychlorinated dibenzo- <i>p</i> -furan; furan
ppm	Parts per million
SWHS	Seveso Women's Health Study
TCDD	2,3,7,8-Tetrachlorinated dibenzo- <i>p</i> -dioxin; most toxic dioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
T4	Thyroxine; thyroid hormone
T3	Thyroid hormone
TSH	Thyroid stimulating hormone

## 1 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are well-known environmental toxicants and endocrine disruptors. PCDDs, PCDFs, and planar PCBs are often grouped together as 'dioxins' or 'dioxin-like compounds', because of their common mode of (toxic) action via Ah-receptor binding.

Humans, at the top of the food chain, are exposed to relatively high amounts as a result of bioaccumulation.

PCBs are a mixture of congeners that can alter the endocrine system by a variety of mechanisms. Different congeners, or structurally similar compounds, may cause different effects. Phenobarbital-like, estrogenic, and neurotoxic effects have been described. Effects on thyroid hormones and hypothalamic cells, resulting in increased levels of gonadotropin releasing hormones, have also been described [1,2].

Various studies have examined the effects of perinatal exposure on the development of humans; some of these study cohorts were exposed to high concentrations as a

result of chemical accidents, the most important accidents being the Yusho, Seveso, Yucheng and Agent Orange incidents.

The Yusho incident took place in the western part of Japan in 1968. Following the accidental leakage of PCBs and PCDFs into rice oil destined for human consumption, a disease appeared, characterised by acne-like eruptions, pigmentation of the skin, and eye discharge. The disease was named Yusho (Oil Disease). More than 1800 patients have been registered as having Yusho and around 300 are deceased [3,4].

Similarly to Yusho, about 2000 people on Taiwan Island ingested rice oil contaminated with PCBs and PCDFs in 1978. The exposed population developed chloracne, hyper-pigmentation, peripheral neuropathy and other symptoms, which were later called Yucheng Disease [5,6].

In 1976 in Seveso, Italy, an explosion at a herbicide manufacturing plant took place. About 30 kg of 2,3,7,8-TCDD, the most toxic dioxin, was released into the environment by the explosion. This resulted in major health consequences, including malformations, miscarriages, cancer and death, for the exposed adults and their children [7,8].

The Agent Orange incident took place between 1962 and 1972. More than 12 million gallons (approximately 54 million liters) of a herbicide (defoliant) named Agent Orange (2,4,5-T) was sprayed over lush South Vietnam, by the US Army during the Vietnam War, with the intention of defoliating forests in order to prevent enemy troops to hide in the forest. However, Agent Orange was contaminated with 2,3,7,8-TCDD. Agent Orange exposure appears to show a significant dose-response relationship with amongst others, a history of benign fatty tumors, chloracne, skin rashes with blisters, and photophobia [9].

Yet, background concentrations, concentrations that average individuals in Europe and the US are exposed to daily have also been related to various negative health effects.

Several animal studies on the reproductive consequences of dioxins have been performed. They suggest that TCDD may affect ovarian function directly or indirectly via the pituitary gland, and thereby influence the menstrual cycle. Abnormalities of the ovulatory cycle and reproductive organs have also been reported [10,2,11]. In a study on monkeys, dioxin exposure was correlated with an increased incidence of endometriosis [12]. Endometriosis is the presence of endometrial glands and stroma outside the uterine cavity, which is often associated with infertility, dysmenorrhea, dyspareunia, and pelvic pain [13].

In this review, the most important results of studies evaluating the relationship between exposure to dioxin-

like compounds and PCBs and reproductive end-points in humans (sex-ratio, menstrual cycle characteristics, endometriosis, sperm quality and prematurity) are discussed.

## 2 Materials and methods

A literature search was performed in Pubmed for references to PCB, PCDD and PCDF exposure and reproductive development. MESH terms used included 'reproduction', 'sex ratio', 'endometriosis', 'menstrual cycle', 'menstruation', 'semen', all in combination with the MESH terms 'PCB', 'PCDD', 'PCDF', 'furan', and 'dioxin'. We focused exclusively on human studies available to the broad public via medical literature. All the articles found by this search, and linked 'related articles' were considered. Publications related to abnormalities caused by other disturbances than PCBs or dioxins were excluded. Articles related to exposure to PCBs or dioxins considering other endpoints than the reproductive system were also excluded.

The results from the various articles were compared. For determination of the effects on reproduction caused by PCBs and dioxins, the sex-ratio, menstrual cycle abnormalities, endometriosis, sperm quality and prematurity were used as end-points.

## 3 Results and discussion

### 3.1 Sex-ratio

The number of X- and Y- bearing sperm in the human ejaculate is usually equal. The normal primary male-to-female ratio (sex-ratio) at conception is presumed to be higher than 1:1. The secondary sex-ratio in Western Europe (at birth) is about 102-103 males to 100 females (sex-ratio of 0.513) [14]. Eleven studies evaluated the influence of PCB and dioxin exposure on the sex-ratio of offspring from exposed parents.

After the incident in Yusho, a study of the sex-ratio (male-to-female ratio) was performed at two PCB/PCDF-affected locations, Fukuoka and Nagasaki. Eighty-five live births were recorded in these regions after the incident. The study showed that the probability of having a male live birth was not significantly different from that of the general population (sex-ratio: 0.513). In one of the Yusho locations, Nagasaki, there were more live girls born alive in the 11 months immediately after the incident, but the probability of a boy born alive did not change significantly

[15]. In contrast, in Mocarelli's Seveso cohort it was observed that between 1977 and 1984 only 26 boys and 48 girls were born in dioxin-exposed families [16].

Another follow-up study on the Seveso cohort, of the children of 239 men and 296 women born from 1977-1996 (offspring: 346 girls and 328 boys) was conducted. The sex-ratio of offspring of exposed mothers as opposed to unexposed mothers was lower (0.545 and 0.608, respectively). The exposed men (with a serum concentration higher than 15 pg/g serum lipids) showed a significantly lower sex-ratio in their offspring as compared to non-exposed men (0.436 as opposed to 0.608). When both parents were exposed, the sex-ratio was not significantly different from the results from solely exposed men (0.442 and 0.436, respectively). It appeared that the highest exposed fathers had a lower sex-ratio than the lowest exposed (0.383 versus 0.434), in other words a dose-effect relationship. Another interesting feature was the fact that men exposed before the age of 19 appeared to be more affected in their offspring's sex-ratio compared to men exposed at later age (0.382 vs 0.469) [17].

The Yucheng cohort showed contrasting results. In one study, the gender ratio was not different between offspring born to 74 Yucheng women, having a live child born during or after the poisoning, and control women [18]. In the second Yucheng study, exposed Yucheng men were found to have a lower percentage of male offspring than unexposed controls. This study cohort consisted of 2061 people who had ingested cooking oil contaminated with heat-degraded PCBs, PCDFs, and PCDDs. The latter study revealed that men exposed before the age of 20 years had a particularly lower chance of having a baby boy than controls. The sex-ratio of offspring of men exposed before the age of 20 was 0.458 as opposed to 0.541 of fathers exposed after the age of 20 years. The sex-ratio of children from men exposed to PCBs after the age of 20 approached that seen in controls (0.541 vs 0.549). Maternal exposure had no significant effect on the sex-ratio of offspring in this study [19].

In a Russian study, TCDD was measured in 84 pesticide workers, who had worked with trichlorophenol and 2,4,5-trichlorophenoxy acetic acid, contaminated with TCDD, resulting in a high TCDD exposure (240 pg/g serum lipids). The cohort comprised 227 children from 150 male and 48 female workers. The sex-ratio of the cohort was significantly lower than that of the control cohort (0.4 vs 0.512). The sex-ratio after maternal exposure showed no significant difference to controls (0.51 vs 0.512). However, once again paternal exposure resulted in a lower sex-ratio in relation to controls (0.38 vs 0.512) [20].

An US study evaluated whether eating PCB-contaminated fish could lead to a lower sex-ratio in offspring. The cohort comprised 173 mothers and 108 fathers and their final offspring. Levels of the summation of several PCB congeners was compared (in quintiles) to the sex-ratio of the lowest quintile. There was evidence that maternal PCB exposure led to a decreased sex-ratio. The odds of giving birth to a male child, among mothers in the highest PCB quintile as opposed to the lowest, was reduced by 82 % (OR: 0.18; 95 % 0.06-0.59). There was no clear effect of paternal serum PCB concentration on the odds of having a male child. There was some suggestion that levels of paternal exposure to PCB may increase the sex-ratio, but these results were weak and inconsistent. No information about individual PCB congeners in relation to sex-ratio was mentioned. DDE was also measured in this study and no relation was found with sex-ratio [21].

In a Michigan cohort, PCB levels of 101 families were compared with the sex-ratio of their 208 children. It appeared that if the PCB concentrations of the fathers exceeded 8.1 microgram/L serum the sex-ratio was increased, after controlling for maternal PCB levels and paternal DDE levels. However in this study, no congener specified analysis was performed and there was no different analysis for parents exposed before the age of 19 [22].

In another study from the United States (San Francisco), 399 mother/child pairs selected between 1964 and 1967 were examined. A lower male:female ratio was seen with increasing PCB (105, 110, 118, 137, 138, 153, 170, 180 and 187) exposure. It appeared that women with higher SUMPCB levels (of the 90th percentile) had a decrease in relative risk of a male birth by 33% [23].

In an Agent Orange study, current TCDD levels of fathers who worked in US Agent Orange factories were estimated from levels in 1987. Although the difference in TCDD levels of the 259 workers were enormous in comparison to 243 controls (254 pg/g vs 6 pg/g lipids), no relation was seen between estimated paternal TCDD exposure and sex-ratio in the offspring [24].

In a prospective study on 50 US women, concentrations of 3 groups of PCB exposure (estrogenic, anti-estrogenic and total), were correlated to the sex-ratio of the offspring of the mothers. In this study PCB exposures were stratified. For the PCBs with xenoestrogenic activity, there was a higher sex-ratio in the second and third stratification and for the anti-estrogenic PCBs there was a decrease in sex-ratio in mothers with the highest exposure stratification [25].

In study conducted in the Australian state Victoria, levels of (sum) PCBs in breast milk of 200 women

were measured and the sex ratio of their offspring was determined. The sex ratio of the offspring did not differ in women with higher and lower levels of PCBs [26].

Seven of twelve studies showed a decrease in sex-ratio after parental dioxin or PCB exposure. In three of these studies the exposure was paternal and in three maternal.

The studies in Seveso and Yucheng showed that the effect seen after father's exposure was even more significant when the paternal exposure occurred before the age of 20 [17,19].

In contrast to these studies, the Yusho cohort, one Yucheng study, an Agent Orange study and a study in Australia, revealed no change in offspring sex-ratio during the ten years after PCB or dioxin exposure. In the Yusho study, however, the age of the parents at exposure and the individual exposure levels of the parents were not mentioned [15]. Furthermore, the Yusho and the first Yucheng study were performed too soon after the incident to include parents exposed before puberty, and their offspring. The Australian study, and two of the three mentioned US studies, exclusively looked at effects seen with sum PCBs, no congener specific analysis was performed in the above mentioned studies.

It can then be concluded that there is strong evidence that the male/female ratio is decreased in the offspring of parents exposed to dioxins or dioxin-like PCBs, and this decrease is even more significant at higher exposures and with exposure at a younger age. While a dose-response effect could explain the decreasing ratio with increasing exposure level, the reason for a decreasing ratio with decreasing age at exposure is unknown. Exposure to a different mixture of PCBs and dioxins could result in different outcomes on sex-ratio. There is evidence that maternal exposure may alter sex-ratio, depending on the estrogenic or anti-estrogenic character of the exposure, but the evidence is not as strong as for paternal exposure.

It is possible that a toxic exposure during pregnancy might selectively damage male fetuses, resulting in a lower male to female sex-ratio at birth [27], it is also possible that the nestling of especially male fetuses in the uterus is inhibited [28]. The answer may be found in the fact that high gonadotropin and low testosterone levels injure Y-bearing gametes before conception [29,30]. Mocarelli et al suggested that TCDD could induce epididymal impairments or act on an Ah receptor, which mediates the effect of change in sex-ratio [17]. Finally, we would postulate that the greater effect seen in males with a younger age at exposure may be the result of damage in a still developing male reproductive system.

### 3.2 Menstrual cycle

Eight studies on menstrual (cycle) abnormalities in humans in relation to PCB and dioxin have been published.

In 1993-1994, 356 Yucheng women were interviewed about their reproductive functions. The data were compared with 312 neighborhood controls. Of the Yucheng women, 16% reported abnormal menstrual bleeding compared to 8% of control women. Stillbirths since 1979 (one year after the PCB/PCDF exposure) was reported as 4.2% in the Yucheng women versus 1.7% in controls. Other reproductive characteristics like menses, fertility, frequency of intercourse, and age at menopause appeared unaffected. More Yucheng women reported childhood deaths than controls (10.2% vs 6.1%) and 7% of the Yucheng women (vs 2% of controls) chose to limit childbearing because of health problems [31]. In a later study on the Yucheng women, the PCB/PCDF exposed women out of a group of 445, had a shorter menstrual cycle length (0.5 days  $p=0.03$ ). This effect was more obvious among premenarcheal exposed women, who also had a longer menstrual flow (0.5 day,  $p=0.04$ ). Women who developed skin lesions after the Yucheng accident had a 1.2 days shorter cycle. There was no higher incidence of menstrual cycle irregularity or dysmenorrhoea [32].

In another study, 301 Seveso women were interviewed 20 years after the explosion. Serum TCDD was also analysed. Among women who were pre-menarcheal at the time of the explosion, higher serum TCDD levels were associated with longer menstrual cycles and reduced odds of scanty flow. It appeared that a 10-fold increase in TCDD levels was associated with an increase of 0.93 days in menstrual cycle length. However, among women who were post-menarcheal at the time of the explosion, no relation was found between serum TCDD and days of menstrual flow and reported scanty flow. In both groups, however, an inverse association with irregular cycles and serum TCDD levels was found [33].

Mendola reported a shortening of the menstrual cycle in association with increasing PCB exposure; exposure arising through ingestion of fish from the highly contaminated Lake Ontario. Women in the cohort were interviewed by telephone about their menstrual cycle and PCB exposure was calculated by duration and frequency of fish consumption at cohort enrolment two years previously. Consumption of more than one fish meal per month was correlated with significant cycle length reductions of 1.11 days. Women who consumed contaminated fish for 7 years or more also appeared to have shorter cycles (0.63 days shorter) [34].

A Japanese study of 81 Yusho women assessed menses 2 years after the accident. 58 % of the women reported irregular menstrual cycles [35,33].

In another study 12 PCBs and  $p,p'$ -DDE were determined in blood samples collected during the third trimester of pregnancy of 2314 pregnant women. The women, participating in the Collaborative Perinatal Project, were enrolled in the 1960s in 12 centers in the United States. Information about usual (pre-pregnancy) menstrual cycle length, regularity, bleeding duration, and dysmenorrhoea was collected. The mean PCB concentration in serum was 3.1  $\mu\text{g/L}$ . After adjustment for confounding factors the total serum PCBs were positively associated with an increasing menstrual cycle length of 0.7 days. Irregular cycles were slightly more frequent amongst those in the two highest categories of PCB exposure. There was also some evidence of an association with DDE. The strengths of these associations increased with various exclusions made to decrease potential misclassification of the outcome and the exposures. There was little evidence for associations between DDE or PCBs and bleeding duration, heavy bleeding, or dysmenorrhoea [36].

In a study on 50 South East Asian immigrant women of reproductive age, 10 PCB congeners, DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and DDE were measured in serum. Daily urine samples were assayed for metabolites of estrogen and progesterone, and the women's menstrual cycle parameters were assessed. It appeared that with each doubling of the DDE level, cycle length decreased by 1.1 days (and luteal phase length decreased by 0.6 days). PCB levels showed no association with cycle length or hormone parameters [37].

In another Taiwanese study on Yucheng girls aged 13-19 years (offspring from exposed mothers,  $n=17$ ), a shorter duration of bleeding per cycle was seen, compared to unexposed children (5.5 days vs 6.5 days,  $p=0.006$ ). The menstrual cycle was also shorter in Yucheng girls ( $p=0.032$ ). There was a higher rate of irregular menstrual cycle in exposed girls, as opposed to controls ( $n=16$ ,  $p=0.018$ ). The irregularity and duration of bleeding during the menstrual cycle was reported using daily records. No difference in dysmenorrhoea rates were found between the groups. In this study serum levels of estradiol and FSH were higher in exposed girls compared to controls [38].

In a study on 119 healthy Taiwanese women placental dioxin TEQ levels were higher in women with irregular menstrual cycles ( $p=0.032$ ). Placental PCB TEQ levels were higher in women with menstrual cycles longer than 33 days ( $p=0.006$ ) [39].

In eight of nine studies, menstrual abnormalities were associated with PCB or dioxin exposure. However,

dissimilar results were found in these studies. In three studies, PCB and TCDD were associated with longer menstrual cycles, while in three studies an increase in PCB/PCDF exposure was associated with a shorter cycle. The serum PCB concentration, however was not directly measured in one study, but calculated by duration and frequency of fish consumption. In two other studies, no association was reported between PCB exposure and menstrual cycle length.

Three studies showed a higher incidence of abnormal menstrual bleeding with higher PCB exposure, while one study showed a decreased incidence of abnormal menstrual bleeding with higher TCDD exposure. A higher rate of an irregular menstrual cycle in exposed women was seen in four studies.

Concluding, the results appear somewhat contradictory amongst the studies, indicating either no effect of exposure, confounding factors or simply inter-subject and/or inter-study differences. The difference in study designs could also be a possible reason for the contradictory outcomes. Additionally, exposure to different kind of congeners may exert different endocrine responses. Timing of the exposure (before or after puberty) probably also plays a role.

A limitation of most of the studies is the fact that menstrual cycle characteristics were mostly based on retrospective self-reporting, rather than on diary data or hormonal measurements.

Another limitation was that no difference was made between DL-PCBs and other PCBs.

It is possible that PCBs and dioxins exerted a direct effect on the hypothalamic cells [2]. It is also possible that these compounds affect thyroxin homeostasis, leading to prolonged duration of menses or even oligomenorrhoea and decreased menstrual flow in hyperthyroidism [40].

### 3.3 Endometriosis

Endometriosis is a gynaecological disorder which is characterised by the presence of both endometrial glands and stroma outside the uterus [13]. Endometriosis is thought to affect 7-10 % of women in the general population [41,42]. Random biopsies at laparoscopic sterilisations have revealed evidence of endometriosis in approximately 25% of women [43]. The exact aetiology of this disease is unknown [44], but several hypotheses exist. One is the premise that retrograde menstruation results in endometrial seeding of the peritoneal cavity. A reduced sensitivity of endometriotic cells to apoptosis could promote the dissemination and implantation of these cells to ectopic sites [45].

Evidence from animal studies suggests that endometriosis is associated with exposure to TCDD and dioxin-like PCBs [44]. We found thirteen studies on this subject.

A study of the TCDD-exposed Seveso women assessed whether dioxin exposure was related to endometriosis in humans. The cohort included 601 participants who were under the age of 30 years at the time of the accident. Complaints of pelvic pain were evaluated, as well as not being able to fall pregnant within a year of trying to. Endometriosis was evaluated during pelvic surgery, by trans-vaginal ultrasound and/or pelvic examination. Surgery was not always performed for endometriosis. Women with serum dioxin levels of 100 pg/g serum lipids or more showed a doubled, but not statistically significant, risk for endometriosis. No clear dose-response was observed [46].

A German study compared serum PCB levels (138, 153, and 180) in 28 cases of endometriosis as opposed to 441 controls. The women with endometriosis had significantly higher PCB levels than their controls [47].

A Belgian study focused on the relationship between exposure to dioxins and PCBs and the occurrence of endometriosis in infertile females. This study group consisted of 42 infertile endometriosis cases and 27 mechanically infertile controls. Exposure to dioxin-like compounds and non-planar PCBs was determined. While the total TCDD equivalent (TEQ) levels were higher in the endometriosis group (median 29 pg/g) compared to the controls (median 27 pg/g), the difference was not statistically significant [48]. In another Belgian study, concentrations of 17 PCDD/F and 16 PCBs in the serum of 10 women with endometriosis were no different from those of 132 controls [49]. In the study of Heiler et al, 71 Belgian women were examined. Among these women, 25 were suffering from peritoneal endometriosis, 25 had deep endometriotic nodules, and 21 were control. Endometriosis was excluded in the controls by questionnaire, pelvic examination, vaginal echography and CA-125 levels. PCDDs, PCDFs and dioxin-like PCBs were measured in the serum. Serum TEQ levels were significantly higher in women suffering from endometriosis in this study [50]. In another study, 22 Italian and 18 Belgian women aged 18-40 years were enrolled to undergo laparoscopy. Twelve Italian and eleven Belgian women had histologically proven endometriosis. PCDDs, PCDFs and dioxin-like PCBs were measured in blood samples of these women and their controls. No significant differences were found in PCDD, PCDF and/or dioxin-like PCB body burdens between the cases and controls. However, significantly higher levels were found in the blood of the Belgian women. Bearing in

mind the higher incidence of endometriosis in Belgium, it is possible that exposure to these chemicals is related to the development of endometriosis [51].

One US control study on 251 women with endometriosis and 538 controls did not find significant relations with estrogenic or SUMPCBs in serum levels [52].

An Israeli study of 44 infertile women with endometriosis and 35 controls with tubal infertility also evaluated a dioxin link. Of the 44 women with endometriosis, 8 had detectable serum TCDD concentrations (18%). Of the controls however, only 1 had detectable TCDD levels (3%). This statistically significant difference was not attributable to higher triglyceride concentrations (which give a higher solubility of dioxin in blood) [53].

In contrast, Boyd et al reported similar levels of PCDDs and PCDFs in 15 women with endometriosis and their control group (15 women). However, the absence of disease was not confirmed in the control group [54,55].

In another study, cases and controls were selected among pre-menopausal women aged 18-50 who underwent laparoscopy for pelvic pain, infertility, or tubal fulguration. Fourteen PCBs and 11 chlorinated pesticides were determined in their serum. Age, body mass index, number of children, and the indication for laparoscopy were controlled for. No statistically significant differences were seen between cases and controls with regards to mean concentrations of the various organochlorines. There was also no significant linear trend between endometriosis and increasing organochlorine concentrations [56].

Buck Louis et al studied a group of 84 women aged 18-40 who were scheduled for diagnostic laparoscopy. Their diagnostic outcomes were compared to 62 congeners of PCBs measured in their serum. Of the 84 women, 32 were diagnosed with endometriosis. As expected, women with endometriosis had fewer pregnancies and live births than their unaffected peers. Gravidity and cigarette smoking were both associated with a significantly reduced risk of endometriosis. Total serum PCBs ranged from 0.5 to 5.3 ng/g serum lipids in women with endometriosis, and from 0.2 to 5.6 ng/g serum lipids for those without endometriosis. The sum of four anti-estrogenic PCB congeners (105, 114, 126, 169) ranged from 0.4 to 1.7 ng/g serum lipids and 0.07 to 0.9 ng/g serum lipids serum, for women with and without endometrioses, respectively. A significant, almost four-fold increase of endometriosis for the sum of the four PCBs with anti-estrogenic properties was observed. The association remained significant at 3.3 after controlling for gravidity, current cigarette smoking, and serum lipids [57].

In an Italian study, 80 women of reproductive age and suspected of endometriosis underwent laparoscopy. Forty had laparoscopic and histologically confirmed endometriosis and the other 40 women had other (benign) gynecological conditions. Higher (sum) PCB levels in the blood were found in women with endometriosis (410 ng/g vs 250 ng/g;  $p=0.003$ ). Levels of separate congeners PCB 118, 138, 153, and 180 were significantly higher in women with endometriosis [58].

In a Belgian study, the mean levels of dioxin-like compounds (DLC) was slightly higher in patients with endometriosis ( $22.3\pm 9.3$  pg TEQ/g lipid) compared to controls ( $20.5\pm 10.8$  pg) using the CALUX method. However, after categorization of patients in a group with 'low' plasma concentrations (<25<sup>th</sup> centile) and a group with 'high' plasma concentrations (>75<sup>th</sup> centile) of dioxin-like compounds, the age-adjusted odds ratio to have endometriosis was 2.44 ( $p=0.04$ ) for women with high concentrations of DLC and it increased to 3.01 ( $p=0.03$ ) when only women with moderate severe endometriosis were considered [59].

In a study of 139 infertile Japanese women who underwent laparoscopy, PCDD/Fs and dl-PCBs were determined in serum. It appeared that the serum TEQ of PCDDs were higher in the control ( $n=81$ ) women than in the cases ( $n=58$ ). Other TEQ values did not differ between control and cases [60].

In a study of the Michigan cohort, women with self-reported endometriosis ( $n=79$  of 943 women; 37 diagnosed by laparoscopy) underwent evaluation of their PBB and PCB exposure. The serum levels were measured in the late 1970's. The PCB exposure was determined using Aroclor 1254 levels. Increased incidence of endometriosis was suggested in women with moderate and high PCB levels, compared to women with low PCB exposure. No higher incidence of endometriosis was seen in women with higher PBB exposure [61]. In another study from the United States (Atlanta) serum levels of PCBs and dioxins of 60 women with endometriosis diagnosed by laparoscopy were compared with 30 controls. In this study no significant relation was found. [62]

Rayon tampons contain dioxins, as a result of chlorine bleaching, and this has been a matter of concern with regards to dioxin exposure and endometriosis. Although the amount of dioxin-like material available from tampons is at least six orders of magnitude lower than estimated daily food exposure levels to these contaminants, we must remember that a local effect may be induced [63,64].

Nine of sixteen studies detected higher PCB or dioxin exposure in women with endometriosis. One study however found higher PCDD TEQ levels in control women

as compared to the subjects [60]. Yet again the study designs were remarkably different and the measured endocrine disrupters in serum were limited. Additionally, definitive epidemiological investigation of endometriosis is difficult, since it requires visual inspection of the pelvis during surgery and pathohistological examination of the tissue. Not all studies excluded endometriosis in the control group. It is interesting to note that, while growth of endometrial cells depends on estrogen [65], Louis et al showed an elevation of anti-estrogenic PCBs in serum of women with endometriosis [57]. In a letter to Nature however, estrogen-like activity in dioxin-like compounds was established [66].

### 3.4 Semen quality of men exposed to PCBs and dioxins

Seven studies considering sperm quality in relation to PCBs or dioxins were found.

In 1998 young men born to Yucheng victims were medically examined for reproductive development, and their semen samples were analyzed. Semen was analyzed in 12 Yucheng young men (sexually mature, aged 16-20 years) and 23 controls. The sperm in exposed (to PCB and PCDF) men showed significantly increased abnormal morphology (37.5% versus 25.9 %), and reduced motility. Another disturbing finding considering the sperm quality is the reduced hamster oocyte penetration by sperm of the exposed men (65.8% versus 73.5 %). The semen volume and sperm count were no different between exposed and unexposed young men [67].

In a 2001 study, the role of environmental estrogens like PCBs (and in this study also the phthalate esters) were evaluated in relation to the deterioration of semen parameters in men who were infertile without obvious etiology. Semen was analysed and PCB concentrations were measured in 21 infertile men and 32 controls (with evidence of conception). PCBs were found in the seminal plasma of infertile men, but not in that of controls. Increasing seminal PCB concentrations showed a significant negative effect on the ejaculate volume, total progressive motility, sperm vitality and sperm osmoregulatory capacity. There was a significant positive correlation between seminal PCBs and the percentage of single-stranded DNA in the sperm, but no significant correlation between PCBs and sperm count, rapid linear progressive motility, percentage of head defects, or the percentage of sperm nuclear chromatin decondensation. It was concluded that PCBs may be instrumental in the deterioration of semen quality in men suffering infertility without an obvious etiology [68].

In Dallinga's study of men with very poor semen quality and men with normal semen quality, blood was examined for hexachlorobenzene (HCBs), *p,p'*-DDT, *p,p'*-DDE, PCBs (118, 153, 138 and 180) and PCB metabolites. No significant differences in PCB levels were found between the groups. However, a significantly decreased sperm count in relation to an elevated PCB metabolite level in serum within the group of men with normal semen quality was found [69].

This was similar to Hauser's study of 29 men, without explicit exposure to PCBs (or *p,p'*-DDE). Most of the recruited men were partners in couples undergoing medical evaluation for an inability to conceive a pregnancy. After examination of the sperm, it appeared that three participants had sperm concentrations below 20 million/mL, seven had less than 50 % motile sperm, nine had less than 4% normal morphology and six participants were below normal in more than one semen parameter. Eighteen participants were normal for the three semen parameters and formed the control group. Higher concentrations of PCBs 118, 138, 153 and  $\Sigma$ PCB were seen with reduced sperm concentrations, and abnormal motility and morphology in all three semen parameters. These data suggest an association between PCBs and abnormal motility, sperm concentration, and morphology [70].

In a study on Agent Orange veterans, who were exposed to TCDD, no relation was found between serum TCDD levels and sperm count or sperm abnormalities [71]. In a 1984 study, no epidemiological proof was seen that exposure to dioxin contaminated Agent Orange amongst Vietnam Veterans resulted in impotence, sterility, miscarriages and birth defects [72]. At follow-up four years later Agent Orange exposure was not associated with difficulty in conception and time to conception of the first child [73].

In a multinational study (Poland, Greenland, Ukraine and Sweden), 319 men underwent an evaluation of semen quality, and of their levels of dioxin-like compounds using CALUX. No evident associations were seen between dioxin-like compound levels and sperm quality [74].

A study of 135 Seveso males, with high TCDD exposure, stratified into three age groups, evaluated sperm quality. The TCDD levels were determined in 1976. The semen quality and reproductive hormone samples collected in 1996 were compared with 184 controls. The younger exposed men ( $n=71$ ; aged: 22-31 yr; mean age at exposure 6.2 years) showed reductions in sperm concentrations ( $p=0.025$ ), percent progressive motility ( $p<0.001$ ), total motile sperm count ( $p=0.018$ ), estradiol ( $p=0.001$ ), and an increase in FSH ( $p=0.055$ ). The men exposed during puberty ( $n=44$ ; aged: 32-39 yrs; mean age at exposure: 13.2) showed an increased total sperm count ( $p=0.042$ ), total motile sperm count ( $p=0.036$ ), FSH (0.038), and reduced

estradiol ( $p < 0.001$ ). No effects were observed in the older group ( $n = 20$ ; 40-47 yrs; mean age at exposure 21.5 yrs). An explanation for the conflicting results was that the Sertoli cell counts differ in each period of age. TCDD may have different effects on androgen and FSH action in infancy than in puberty [75].

In another study 39 sons (born between 1977 and 1984) from mothers exposed to dioxins after the Seveso accident were examined. A total of 21 breast fed sons of exposed mothers had lower sperm concentration ( $p = 0.002$ ), total count (0.02), progressive motility ( $p = 0.03$ ) and motile count ( $p = 0.01$ ) compared to breast fed comparison. Formula fed exposed ( $n = 18$ ) compared to formula fed controls ( $n = 21$ ) and 36 breast fed controls had no sperm-related differences [76].

Five of the eight studies focusing on sperm quality showed that men with higher serum concentrations of PCBs and/or PCB congeners and/or PCDFs have shown effects on sperm quality. In one study a lower sperm count but normal semen quality were found in men with higher PCB body burden [69].

The two Agent Orange studies also showed no relation between exposure and sterility

The study on Seveso men showed that time of the exposure is very important and different results were seen amongst men exposed before, during and after puberty [75].

The effects seen in the Yucheng, Seveso, and Agent Orange study are of major importance, because these men were exposed to high concentrations of PCBs. In contrast, other studies focused on average populations, i.e. not at increased risk of exposure to PCBs, but on men suffering from sub-fertility or infertility. Whilst the exposures are orders of magnitude apart between the former and latter, some of the effects seen were similar.

The finding of a lower sperm count and abnormal semen quality in men with background concentrations of PCBs is alarming. While bearing in mind the limited cohort sizes, it remains very likely that even background PCB exposures cause a decrease in sperm count and this might explain the dramatic decrease in sperm counts of men in Western Europe over the last generation [77]. The effects of PCBs (and PCDFs) on semen parameters might be the result of hormonal effects of the toxins, since animals exposed to these chemicals exhibit hormone dysfunction and altered sexual maturation [78].

Effect of AhR-signalling may be stimulatory or inhibitory, depending on other factors including level of exposure and developmental period, whereby target cells and key level regulatory molecules may differ [75].

### 3.5 Prematurity

In a meta-analysis within 12 European Birth Cohorts studying PCBs and DDE in cord blood a lower birth weight was found with PCBs, however no relation with gestational age was found [79]. But in samples from the Child Health and Development Studies pregnancy cohort a negative relation of 1-3 days is described in relation with PCB levels measured in maternal *postpartum* plasma [80]. In a review of papers published between 1992 through August 2012 no relation with environmental contaminants and preterm births is found [81]. In Europe however a negative relation with gestational age is found of half a week with dioxin activity measured with the CALUX method in cord blood, particularly in boys [82].

## 4 Conclusion

Summarizing, there is evidence that (paternal) PCB/dioxin exposure influences the sex-ratio of offspring. Effects on menstrual cycle characteristics of these compounds are seen, however the character of these effects are inconclusive, probably an effect of exposure to different congeners of PCBs and dioxins. Endometriosis was in most studies correlated with a higher PCB/dioxin exposure. PCB/dioxin exposure seems to influence semen quality, leading to increased abnormal morphology and reduced motility; however the timing of the exposure (before or after puberty) seems to be crucial in interpreting the observed effects. A shortening of gestational age was related to higher maternal PCBs and dioxin, measured in maternal *postpartum* plasma.

It can then be concluded that health effects on the reproductive system in PCB/dioxin-exposed human populations have been seen, not only in highly exposed cohorts, but also in average populations with background exposures. That background concentrations of dioxin-like compounds and PCBs, in people not living in “contaminated” areas, have effects on the reproductive development is disconcerting and warrants further research.

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**Table 1:** Summary of the mentioned studies

End-point	Author	Cohort	Relevant results
<b>Sex-ratio</b>	Mocarelli et al 1996	Seveso; 26 boys, 48 girls	More female offspring in exposed parents.
	Rogan et al 1999	Yucheng victims: 74 women and their 137 children	No difference in sex-ratio between Yucheng women and control women.
	Mocarelli et al 2000	Seveso; 239 men, 296 woman with their 674 children (346 girls and 328 boys)	Lower sex-ratio with increasing TCDD serum concentrations in exposed fathers. Fathers exposed before the age of 19 produced a lower sex-ratio in their offspring.
	Schnorr et al 2001	US: 258 Agent Orange workers and 245 controls	No relation between paternal TCDD levels and sex-ratio of the offspring
	Yoshimura et al 2001	Yusho; 85 live births	No significant difference in sex-ratio offspring from PCB/PCDF exposed parents and normal population.
	Carmaus et al 2002	US: Michigan: 101 families, 208 children	Higher PCB levels in the fathers was related to a higher sex-ratio in their offspring.
	Ryan et al 2002	Russia: 150 male workers and 48 female workers and their children	Lower sex-ratio was found in fathers with higher TCDD levels.
	Del Rio-Gomez et al 2002	Yucheng victims; 2061 subjects	Lower sex-ratio in offspring of exposed (PCB/PCDD/F) men. Lower sex-ratio in offspring of men exposed before the age of 20.
	Weiskopf et al 2003	US: 173 mothers and 108 fathers and their children	Higher level of PCBs in mothers was related to lower sex-ratio of their offspring.
	Khanjani et al 2006	Australia: 200 mothers and their children	No effects on sex-ratio in women with higher levels of PCBs in breast milk.
	Taylor et al 2007	US; 50 mothers and their children	Higher sex-ratio in women with higher estrogenic PCB levels. Lower sex ratio in offspring of women with higher anti-estrogenic PCBs.
Hertz-Picciotto et al 2008	399 women/ child pairs (1960) and control	Women of the 90 <sup>th</sup> percentile of total PCBs in the serum had a decrease in relative risk of a male birth by 33%	
<b>Menstrual cycle characteristics</b>	Kusuda et al 1971	81 Yusho women (PCB/PCDF exposure)	58% had irregular menstrual cycle abnormalities 2 years after the incident
	Mendola et al 1997	Women living near to lake Ontario (PCB exposure)	Women with higher PCB exposure (more than 7 years consumption of contaminated fish) had shorter menstrual cycle (0.63 days)
	Yu et al 2000	356 Yucheng women (PCB/PCDF exposure) and 312 controls	More menstrual abnormalities, stillbirths and limitation of offspring because of health problems in exposed woman. No relation with fertility, other characteristics of menses, frequency of intercourse or age at menopause.
	Eskenazi 2002	301 Seveso women (TCDD exposure)	Women pre-menarcheal before incident: 10-fold increase in TCDD levels associated with increase of 0.93 days in menstrual cycle length.
	Cooper et al 2005	2314 Women from United States (PCB/DDE exposure)	Serum PCB was positively associated with increase in menstrual length of 0.7 days. More irregular cycles in highest category of PCB exposure.
	Windham et al 2005	50 Asian immigrant women (PCB/ DDT exposure)	No association with PCB exposure and menstrual cycle length.
	Yang et al 2005	17 Yucheng girls aged 13-19 years and 16 controls.	Exposed girls showed shorter mean duration of bleeding per cycle than controls. Higher rate of irregular menstrual cycle in exposed girls.
	Chao et al 2006	Taiwan: 119 women.	Placental dioxin TEQ levels were higher in women with irregular menstrual cycles. Placental PCB TEQ levels were higher in women with menstrual cycles longer than 33 days.

End-point	Author	Cohort	Relevant results
<b>Endometriosis</b>	Yang et al 2011	445 women (Yucheng women and controls)	PCB/PCDF exposed Yucheng women had a shorter menstrual cycle length and a longer duration of bleeding in each cycle.
	Gerhard and Runnebaum 1992	Germany: 28 women with endometriosis and 441 control women	Higher levels of PCBs in women with endometriosis.
	Boyd et al 1995	15 women with endometriosis and 15 controls	Similar levels of PCDD/F in cases and controls.
	Mayani et al 1997	Israel: 44 infertile women with endometriosis and 35 infertile controls	Higher levels of serum dioxin in women with endometriosis.
	Lebel et al 1998	68 women with endometriosis 70 controls	No association between endometriosis and PCB concentration.
	Pauwels et al 2001	42 infertile women with endometriosis and 27 infertile women without endometriosis	Higher TEQ levels in women with endometriosis.
	Eskenazi 2002	Seveso: 601 women (TCDD exposure)	A doubled, non-significant, non-dose-related risk for endometriosis in women with serum levels of 100 ppt or higher.
	Fierens et al 2003	Belgium: 10 women with endometriosis 132 controls	No difference in PCDD/F and PCB levels between cases and controls.
	De Felip et al 2004	22 Italian (12 with endometriosis) and 18 Belgian women (11 with endometriosis)	No difference in PCDD/F and PCB levels between cases and controls. However, higher levels were found in Belgian women, where the incidence of endometriosis is also higher.
	Louis et al 2005	US: 84 women with diagnostic laparoscopy	Higher incidence of endometriosis in women with higher levels of anti-estrogenic PCBs.
	Heillier et al 2005	51 women with endometriosis and 21 controls	Higher serum TEQ levels in women with endometriosis.
	Tsukino et al 2005	139 infertile Japanese women (58 cases, 81 controls)	Higher PCDD TEQ levels in the control group. No differences between TEQ levels in cases and controls.
	Porpora et al 2007	80 Italian women.	Higher levels of PCBs were seen in women with endometriosis.
	Hoffman et al 2007	97 women of the Michigan PBB cohort.	Women with self-reported endometriosis (37 diagnosed by laparoscopy). No higher incidence of endometriosis in women with higher PBB exposure. Increased incidence of endometriosis was suggested in women with moderate and high PCB levels compared to women with lower PCB exposure.
Niskar et al 2009	Atlanta: 60 women with endometriosis, 30 controls	Serum levels of PCBs and dioxins in 60 women with laparoscopically diagnosed endometriosis did not differ significantly from 30 controls.	
Trabert et al 2010	United States: 251 cases and 538 controls	Women with endometriosis had no significant higher estrogenic or SUMPCB levels than control.	
Simsa et al 2010	96 women 106 control (Belgian)	Women with higher levels of DLCs had a significantly higher probability to have endometriosis	
<b>Semen quality and fertility in men</b>	Guo et al 2000	Yucheng: 12 exposed men and 25 controls	Increased abnormal morphology, reduced motility and reduced capacity to penetrate hamster oocytes in sperm of PCB exposed men. Semen volume and sperm count did not differ.
	Rozati et al 2002	India: 21 infertile men and 32 controls	PCBs found in semen plasma of infertile men and not in controls. Significant negative correlation between PCB concentrations and ejaculate volume, total progressive motility, sperm vitality and sperm osmoregulatory capacity. Positive correlation between PCBs and percentage of single-stranded DNA in sperm but not with sperm count, rapid linear progressive motility, morphology.

End-point	Author	Cohort	Relevant results
	Dallinga et al 2002	Netherlands: 34 men with bad sperm quality and 31 men with normal sperm quality	No significant differences in PCB levels were detected in men with poor semen quality compared to men with normal semen quality. However, a significantly decreased sperm count in relation to higher PCB metabolites was found in the control group.
	Henriksen et al 1996	US: Agent Orange veterans	No relation between TCDD serum levels and sperm count or sperm abnormalities.
	Hauser et al 2002	Massachusetts: 29 men presented in Massachusetts General Hospital andrological laboratory	An association was seen between PCBs and abnormal motility, sperm concentration and morphology.
	Toft et al 2007	319 men from Poland, Greenland, Ukraine and Sweden	No relation between dioxin-like compounds in the serum and sperm quality.
	Mocarelli et al 2008	Seveso: 135 males exposed to TCDD, stratified into 3 age groups, and 184 controls	71 men (22-31 yr) showed reductions in sperm concentrations, percent progressive motility, total motile sperm counts, estradiol and an increase in FSH. 44 men aged 32-39 showed an increased total sperm count and total motile sperm count, FSH and a reduced estradiol. No effects were observed in the older group (40-47 years of age).
	Mocarelli et al 2011	39 sons (born 1977-1984) from mothers exposed to dioxin (Seveso incident)	21 breast fed sons of exposed mothers had lower sperm concentration, total count and progressive motility compared to breast-fed comparison.