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Review

Epidemiological evidence on reproductive effects of persistent organochlorines in humans

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Abstract

Organochlorines are widespread pollutants in humans. Concern about adverse reproductive effects of these compounds arises from accidental exposure of humans and experimental studies. Recently, this issue has been addressed by a number of studies of exposed populations and hospital-based case-referent studies. These studies indicate that high concentrations of persistent organochlorines may adversely affect semen quality and cause testicular cancer in males, induce menstrual cycle abnormalities and spontaneous abortions in females, and cause prolonged waiting time pregnancy, reduced birth weight, skewed sex ratio, and altered age of sexual development. However, most effects have been demonstrated at exposure levels above the present day exposure level in European and North American populations. Due to inherent methodological problems in several of the available studies, additional research is needed to fully elucidate the possible adverse effects of organochlorines on human reproductive health.

Keywords: Organochlorines; PCB; DDT; DDE; Reproduction; Fertility; Human; Endocrine disruption

1. Introduction

Organochlorines include a number of anthropogenic compounds manufactured in large scale since 1930s. The most widespread organochlorines in the environment and in human tissues are polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), especially the DDT degradation product dichlorodiphenyldichloroethylene (DDE) (Fig. 1) [1]. The concentration of these compounds and several other organochlorines is highly correlated in serum samples from the general human population, and exposure to the most common PCB congener (CB-153) has therefore been suggested as an indicator of overall exposure to persistent organochlorines [2]. The co-occurrence of these compounds in human samples makes it difficult to establish which compounds cause the observed effects. Moreover, one group of organochlorines, the dioxins, is more toxic than most PCB congeners, and has the same mechanisms of action as the coplanar PCB congeners [3]. This review intends to describe not the detailed, specific mechanisms of action of individual PCB congeners or DDT and metabolite toxicity, but the epidemiological findings of exposures to mixtures of these compounds. Throughout this paper, we focus on PCBs and DDTs, but will report on the observed effects of dioxins and other persistent organochlorines on reproductive outcomes in the studies where they have been measured together with PCBs or DDTs.

The production of PCBs and DDTs has been limited or completely banned since 1970s in most developed countries. The last PCB production facility, located in Russia, was shut down in 1993 [4]. However, organochlorine compounds including PCBs are still being released into the environment by (1) use, disposal or accidental release from previously produced material, (2) volatilization of previously released material, and (3) creation of PCBs and dioxins during combustion processes [4,5]. Furthermore, some developing countries are still using DDT as vector control. The compounds are highly lipophilic ($\log K_{ow} = 4.7–6.9$, [3,6]), and resistant to both biotic and abiotic degradation, which results in long half-lives in both biota and, e.g., sediments. This, in turn, results in considerable bioaccumulation and biomagnification in the food chain.

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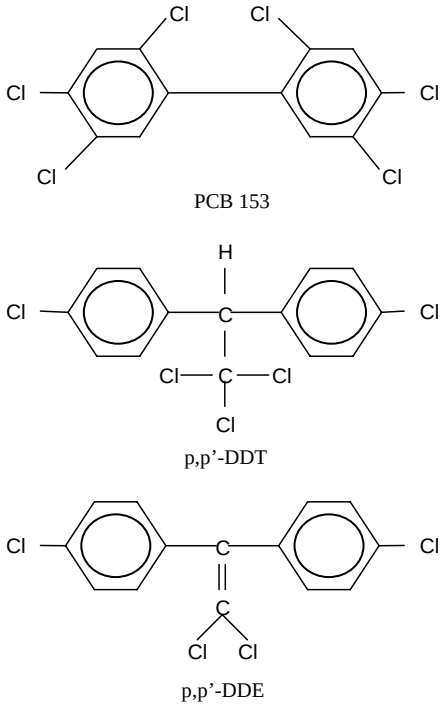

Fig. 1. Structural formula a polychlorinated biphenyl (PCB-153), dichlorodiphenyltrichloroethane (*p,p′*-DDT), and dichlorodiphenyldichloroethylene (*p,p′*-DDE).

The highest concentrations are found in organisms with a long life (including humans) at the top of the food chain.

The concentration of PCBs and DDTs in human tissues has decreased since the ban of these compounds in most countries some 30 years ago, but during the last 10 years the decrease has leveled off [3,7]. The compounds continue to be detected in blood and milk samples from humans all over the world. Furthermore, in areas with present DDT use, high concentrations are still found [8]. These xenobiotics may, therefore, still pose a threat to human reproduction. Moreover, since several PCB congeners and some DDTs and their metabolites have weak hormone- or anti-hormone-like action in in vitro and in vivo assays, evidence on the human reproductive toxicity of these compounds may contribute to our understanding of the environmental hormone hypothesis [9].

The reproductive toxicology of persistent organochlorines has been reviewed previously [1,10–13], but recent advances within the field – especially new epidemiological data – have added information about the potential adverse effects on human reproduction both in highly exposed areas and in the general population. The most recent review [13] is mainly a descriptive study of the effects of PCBs on development and reproduction in human and animal studies. In this review, we critically evaluate recent advances in epidemiological studies of human reproductive disorders related to persistent organochlorines with particular reference to PCBs and DDTs. We also include key studies dating back to 1980 for reference.

2. The literature database

To compile relevant papers for the review, an initial search in Medline (PubMed) for human studies published in English with an abstract and using the search string: (PCB or DDE or DDT) and (fertility or reproduction) produced 408 hits on July 14, 2003. Using the abstracts, we identified original epidemiological studies on reproductive outcomes (time to pregnancy, semen quality, congenital malformations of sexual organs, cancers in reproductive organs, birth weight, gestational age, spontaneous abortion, preterm delivery, pubertal development and early onset of menopause).

Adding secondary references and additional searches on the reproductive parameters provided a total of 62 papers which were included in the primary review. Thirty-seven of the papers were dated year 2000 or later and the earliest paper included was from 1980. The literature database was not restricted by a priori set quality criteria. All papers are summarized in Appendix A.

Potential neurodevelopmental disturbances were not included in this review, but PCBs have been demonstrated to have a marked effect on the neurodevelopment of children (recently reviewed [14]). Furthermore, the inclusion of cancers was restricted to cancers in reproductive organs. The possible associations between PCB, DDE and other cancers, including breast cancer, have recently been covered [15,16].

3. Method for comparison of exposure level among studies

The organochlorine exposure assessments are not complete for all studies, and when measured, different methodologies have been used and the compounds have been measured in different human tissues and secretions. Caution should therefore be taken in direct comparisons among these studies. However, from studies where organochlorine concentration are reported in more than one tissue or secretion, some crude estimates of conversion factors can be made. Hence, the total PCB concentration is about 3.6 times larger than the CB-153 concentration [17] and the lipid basis PCB concentration is about 400 times larger than the plasma wet basis concentration [18]. Furthermore, the concentration in breast milk fat is roughly 150 times greater than in maternal serum and the concentration in cord serum is usually less than 50% of the maternal serum concentration [3]. Similar differences can be assumed for DDE, since the differences are mainly due to differences in fat content and the majority of PCBs and DDTs are bound to fat. Throughout the text PCB and DDT concentrations are given as fresh weight concentrations when not specified, and it is specified when the concentrations are given as lipid-based concentration.

4. Biological capability to conceive (fecundity)

4.1. Male fecundity

A decreasing trend in human sperm counts may have occurred in several European regions during the last 50 years (Paris [19], Scotland [20], Belgium [21], Denmark [22]). A recent study of Danish military conscripts reported a median sperm count close to the value of 40 $\times$ 10^6 ml^{-1} seminal fluid [23], below which increased waiting time to pregnancy may occur [24]. The decrease in sperm count is paralleled by a rise in the trend of testicular cancer and malformations of the male reproductive organs such as hypospadias and cryptorchidism [25]. The causes of these reproductive abnormalities are not known, but since several environmental chemicals are able to interfere with endocrine processes and have been demonstrated to induce similar malformations in laboratory animals [25], it has been suggested that at least some of the reproductive disorders may be due to the increased exposure to chemicals with endocrine active properties, including PCBs and DDTs. A recent hypothesis suggests that male infertility, testicular cancer, cryptorchidism and hypospadias share a common etiology as a part of the so-called Testicular Dysgenesis Syndrome (TDS) [26]. TDS was suggested to be caused by 'endocrine disrupters,' with estrogen and anti-androgen effects impairing early fetal life Sertoli- and Leydig-cell functions, thereby giving rise to the patho-physiological conditions that are part of the syndrome. However, the epidemiological evidence for the 'endocrine disruption hypothesis' has so far been limited and inconsistent [27].

A number of available epidemiological studies on effects of PCB congeners and DDT metabolites on human semen quantity and quality is limited (Table 1). Two accidental episodes of cooking oil contamination with PCBs and PCDFs occurred in Japan in 1968 and Taiwan in 1979. The two episodes gave rise to the so called 'oil-disease,' named the Yusho (Japan) and Yucheng (Taiwan), with approximately 4000 people developing symptoms of intoxication. These well-studied populations serve as reference for the effects of very high exposure to PCBs and PCDFs. Such exposures have been shown to be hazardous for semen function. Twelve of the boys exposed in utero in 1979 to very high doses of PCBs and PCDFs during the Yucheng disaster showed abnormal sperm morphology, reduced sperm motility and capacity to penetrate hamster oocytes as adults, compared with age matched controls [34]. Forty men, postnatally exposed during the Yucheng disaster, were examined and similar changes in sperm function were found, except that the sperm motility was unaffected [35]. The sperm cell concentration was unaffected in both studies.

A study of transformer repairmen revealed no deviant sperm cell concentration at exposure levels associated with a change of thyroid hormones [28]. Exposed workers had a median total PCB concentration in serum of 12 ng/g. Unfortunately, only sperm cell concentration and not other semen quality characteristics were measured in this study, which takes advantage of a three-fold documented exposure contrast between PCB-exposed workers and the reference group.

In a recent study from Mexico where DDT remains in use, a high concentration of *p,p′*-DDE was measured in serum lipids (mean 77900 ng/g) and a negative correlation (*r*

Table 1
Epidemiological studies addressing effects of persistent organochlorines on semen quality

Reference and place	Exposure group/compounds	Semen quality
[29], New York State, USA	Seminal fluid *p,p′*-DDE	→
	PCB	→
	When sperm count <20 million/ml	↓ (motility)
[28], Maryland, USA	PCB in serum	→
[33], Germany	Seminal fluid PCB	↑ (combined index of volume, concentration, and normal morphology)
[34], Taiwan	Boys prenatally exposed to PCBs + PCDF	↓ (normal morphology, motility, and oocyte penetration capacity)
[8], Mexico	*p,p′*-DDE in present use area	↓ (concentration, volume)
[30], The Netherlands	DDTs	→
	PCBs	↓ (concentration and motility)
	In blood of infertility patients	Female factor infertility subgroup only
[31], Massachusetts, USA	PCBs, *p,p′*-DDE	(↓) (motility, concentration and normal morphology)
[32], India	PCB	↓ (motility, volume and vitality)
[35], Taiwan	Adults exposed as teenagers or adult to PCBs + PCDF	↓ (normal morphology, oligospermia rate and oocyte penetration capacity)
[2], Sweden	PCB (CB-153)	↓ (motility)

→: no effects on semen quality; ↓: significant decrease in semen quality (affected outcomes); (↓): reported decrease in semen quality (outcomes) but not significant; ↑: significant increase in semen quality (outcomes).

$= -0.42$) was found between serum lipid concentration of p,p'-DDE and sperm cell concentration in men from the general population not occupationally exposed to DDT. Also the bioavailable testosterone/total testosterone was negatively correlated with p,p'-DDE ($r = -0.47$) [8]. Although only 24 men were included in this study and its findings need to be corroborated, the large range of exposure among individuals reveals important clues as to the effects of this compound on male semen quality.

A recent Swedish study including 305 military conscripts also found a negative correlation between bioavailable testosterone measured as testosterone/SHBG and organochlorine exposure measured as CB-153 ($r = -0.25$) and a small, but statistically significant negative correlation ($r = -0.13$) between sperm cell motility and PCB-153 [2]. However, no effect was observed on sperm cell concentration, testis size, FSH, LH, and Inhibin B level.

Furthermore, studies comparing infertility clients with normal or poor semen quality indicate that PCB and DDE may be negatively related to sperm count and motility. A negative association between PCB concentration in seminal fluid and sperm motility in the subset of men with less than 20 million sperm cells/ml has been reported [29]. However, another study found a negative association between PCB metabolites in blood and sperm count in the subgroup of men with normal semen quality [30]. These findings in subsets of the study population are thus inconsistent and could represent chance findings. Preliminary results from another ongoing study of men referred to infertility make-up [31] suggest increased PCB and DDE serum concentrations in patients with low sperm concentration and motility, but due to the small number of samples collected, proper statistical evaluation cannot be performed. Markedly higher PCB levels were found in infertility patients without any obvious etiology compared with a control group selected to exclude persons with known risk factors for subfertility [32]. Since the same criteria were not used for the case group, these findings may be due to the selection procedure. Moreover, very high detection levels for some PCB congeners cast doubt on the analytical quality. Another study of men investigated for infertility showed no correlation between the spermiogram parameters motility, morphology and density and the concentration of PCB, hexachlorobenzene and gamma hexachlorocyclohexane in seminal plasma [33]. Surprisingly, the concentrations of these compounds were higher in men with normal semen parameters than in those with abnormal semen quality. The authors did not explain this unexpected finding, but the results of studies of men with infertility problems should generally considered with caution. Such groups may represent individuals with different levels of sensitivity to the gonadotoxic effects of chemicals and they may also have other sperm defects (e.g., impairment of sperm DNA integrity) not detected by classical semen parameters.

Several sources of potential bias exist in these studies and the results should therefore be interpreted with caution. In particular, the limited study size in most of these studies precludes detection of minor effects on sperm count and other seminal characteristics with large within- and between-subject variations [36] and the low participation rate in most of these studies may cause the study population to be biased. Furthermore, small effects on motility can be produced by undetected errors related to measurement of motility, which is highly dependent on time from collection to analysis and temperature conditions [37]. Such a measurement error would, however, most likely be non-differential with respect to exposure, and therefore make it more difficult to detect true associations between exposure and sperm motility. Larger studies with a high participation rate are therefore needed to elucidate the possible adverse effects of organochlorines on semen quality. Furthermore, such surveys should be based on cohorts derived from the general population rather than from men coming for infertility investigation. New markers of semen quality like, e.g., sperm DNA integrity should also be added to the list of end points in order to disclose significant changes in the fertilizing capability of the male gametes not disclosed by classical semen parameters.

4.2. Female fecundity

Infertility in a couple can be ascribed to male factors in 30–40%, female factors in about 50% and to either both male and female or unknown factors in the remaining 10–20% of the cases [38].

The primordial follicles are formed right after birth, and remain in the ovary in this inactive resting stage until 12 weeks before ovulation. The primary formation of follicles can therefore only be disturbed by exposure in the early life stages, but several processes involved in the maturation of the oocytes may be sensitive to chemical disturbances, especially through interference with hormone regulation in the hypothalamus pituitary gonad axis [39]. Early onset of menopause has been interpreted as a marker of damage to the follicular pool, whereas irregularities of the menstrual cycles are often caused by disturbances of follicular maturation or hormonal disturbances.

The effects of organochlorine exposure on natural menopause have been studied in a recent American cohort study [40], where a hazard ratio of early onset natural menopause of 1.4 (95% CI = 0.9–2.4) was seen for the 10% with the highest measured exposure to p,p'-DDE, whereas no effect of PCBs was indicated. In a follow-up study of the Yucheng cohort [41], the age at onset of menopause was not affected in the women exposed to high concentrations of PCBs before the age of 45 years. However, in the same study an increased fraction of the PCB-exposed women experienced abnormal menstrual bleedings compared with age-matched neighborhood controls. Also, in a study of sports fishermen families from New York State, menstrual

irregularities (decreased cycle length) were correlated with PCB exposure measured as fish consumption [42].

Other reproductive abnormalities have been correlated with serum organochlorine levels in infertility patients. Especially endometriosis has been associated with high concentrations of PCBs and a decreased conception rate was observed among women with elevated DDT exposure [43]. However, the results of this study should be interpreted with caution since the study population consisted of a selected fraction of the population and the explorative analyses could, by chance, have detected spurious associations. Other case-control studies revealed no association between organochlorine exposure and endometriosis [44,45].

4.3. Couple infertility

A direct and functional measure of a couple's fertility is the time to pregnancy, which is defined as the time from a couple is starting to have unprotected sexual intercourse to the onset of pregnancy. The time to pregnancy is only defined in couples that discontinue regular contraception to get children or in couples abstaining from sexual relations before they decide to have a child. Bias because of incomparability of sexual behavior in the study groups is one important limitation of time to pregnancy studies [46]. In clinical settings, infertility is often defined by a time to pregnancy exceeding 12 months. Even a small fraction of healthy sexually active couples is expected to have delayed conception because of 'bad luck.' Time to pregnancy does not indicate if a delayed conception is caused by male or female factors. Moreover, an extremely strong toxicant causing sterility would not be detected by the usual design of time to pregnancy studies which only includes couples that have achieved a pregnancy. However, it can be detected if all women having ever tried to become pregnant are considered eligible for study entry.

The issue of PCB exposure via fish consumption has been addressed through questionnaire surveys of the time to pregnancy in three cohorts, namely anglers from New York State or Lake Michigan and Swedish fishermen's wives and sisters (see Table 2). In all cohorts the authors report some indications of increased time to pregnancy in relation to PCB exposure, although the reports are not entirely consistent. In the New York State angler population, a decreased conception success was observed among women eating fish from polluted lakes more than once per month or who had been consuming fish for more than 3 years [47], but the fathers' fish consumption did not seem to contribute to the decreased conception success [48]. The opposite tended to occur in the Michigan cohort with an increased proportion of males with a TTP >12 months among sports fishermen with a high consumption of sports fish, but no difference in experienced periods of infertility among their wives [49]. When the New York State data were analyzed with linear regression analysis or using a dichotomous outcome (TTP less or greater than 12 months), no association between fish consumption and conception delay could be detected [50,51]. However, the former approach evaluating fecundability rates by discrete Cox regression is considered the most appropriate [52]. The Swedish fishermen's family study revealed a decreased conception success of the east coast fishermen's wives who ate fish from the polluted Baltic Sea compared with the west coast fishermen's wives. The decreased conception success was mainly observed in the subgroup of heavy smokers among the east coast fishermen's wives as compared with heavily smoking fishermen's wives from the west coast [53]. However, when TTP of the Swedish fishermen's sisters were studied, no difference between east coast and west coast cohort affiliation or fish consumption could be observed; on the contrary, the results suggested beneficial effect of fish consumption on TTP [54]. All three cohorts lack direct documentation and quantification of exposure measures. The exposure was simply estimated from self-reported past fish consumption. Subsequent exposure measures (CB-153 measurements in serum) in the subgroup of Swedish east coast fishermen's wives [55], the conception success was not significantly different between groups of the population with different exposures. However, the blood samples for PCB analysis were drawn 1–36 years after the pregnancies occurred and the PCB concentration around the time of conception was modeled from the present level.

A recent study indicated that maternal exposure to DDT caused a prolonged time to pregnancy of their daughters (32% decrease in the probability of getting pregnant within each cycle per 10 ng/g increase in *p,p′*-DDT in maternal serum), whereas DDE exposure was associated with a shorter time to pregnancy (16% increase in the probability of getting pregnant per 10 ng/g increase in *p,p′*-DDE in maternal serum) [56].

Due to the uncertainties associated with the exposure estimate in most of the TTP studies and the inconsistency of the results, no firm conclusions can be drawn about the possible adverse effects of organochlorine exposure on time to pregnancy.

5. Cancer in reproductive organs

5.1. Testicular cancer

A new Swedish study found the levels of the organochlorines PCBs, HCB, *trans*- and *cis*-nonachlordane, but not *p,p′*-DDE to be increased in the mothers of 44 testicular cancer cases compared with age-matched controls, but only *cis*-nonachlordane was significantly increased in the cases themselves [57], suggesting that exposure during the fetal or neonatal stage (lactational) to organochlorines increased the testicular cancer risk. However, the organochlorine level in the mothers was measured at the time of testicular cancer onset in their sons (mean age 30 years), and could be markedly altered after the pregnancy.

Table 2
Epidemiological studies addressing effects of persistent organochlorines on time to pregnancy

Reference and place	Exposure group/compound	Result		TTP	Adjusted for
[50], New York State, USA	PCB exposure fish consumption; duration of fish consumption	Regression analysis TTP: β-coefficients small and insignificant		→	Smoking, gynecologic history, STDs
[48], Michigan, USA	Paternal PCB estimated from fish consumption	Risk of conception delay OR (95% CI) (TTP >12 months)			Female age, smoking, education and income
	None	1.0 (referent)			
	<1 mg	0.68 (0.29–1.56)			
	1–7 mg	1.45 (0.71–2.93)			
	>7 mg	0.49 (0.18–1.33)		→	
[49], Michigan, USA	Sports fish consumption 1993–1995	Risk of infecundity (TTP >12 months) OR (95% CI)			Religion, age, race, education, income, smoking, alcohol, partners fish consumption
		Male	Female		
	None	1.0	1.0		
	Low	1.4 (0.5–3.9)	0.8 (0.4–1.9)		
	Medium	1.8 (0.6–5.0)	0.8 (0.4–1.8)		
	High	2.8 (1.0–8.0)	1.0 (0.4–2.4)	↑ (male)	
[47], New York State, USA	Maternal PCB estimated from fish consumption	Conception success (95% CI)			Age, smoking, gynecologic history, gravidity and history of fertility drugs
	None	1.0			
	<1 mg	0.82 (0.65–1.04)			
	1–7 mg	0.77 (0.52–1.11)			
	>7 mg	0.87 (0.47–1.43)		→	
	However significant reduction when				
	Duration of fish exposure 3–6 years	0.75 (0.59–0.91)		↑	
	Or number of monthly fish meals >1	0.73 (0.54–0.98)		↑	
[53], Sweden	Swedish fishermen's wives	Conception success RR (95% CI)			Unadjusted. (Adjustment for age, year of birth, average work hours, smoking and parity gave similar results)
	West coast	1.0			
	East coast	0.86 (0.75–0.99)		↑	
	Fish consumption within east coast cohort				
	None	1			
	>2 meals per month	1.07 (0.90–1.27)		→	
[51], New York State, USA	Fish consumption as PCB exposure index	Risk of infecundity (TTP >12 months) OR (95% CI)			Age, smoking, female angler status, gynecologic history and education

	Fish meals/month	Resolved	Unresolved		
	None	1.0	1.0		
	≤1	0.66 (0.28–1.53)	0.88 (0.40–1.97)		
	>1	1.24 (0.59–2.57)	1.27 (0.64–2.53)	→	
[55], Sweden	Swedish east coast fishermen's wives. PCB (CB-153) estimated at time of pregnancy from 4 to 20 years back in time	Conception success RR (95% CI)			Unadjusted (potential confounders only minor effects on estimate)
	Low 37–206 ng/g lipid	1			
	Medium 207–330 ng/g lipid	0.77 (0.47–1.28)			
	High 331–1036 ng/g lipid	0.95 (0.74–1.23)			
[54], Sweden	Swedish fishermen's sisters	Conception success (95% CI)		→	
	East coast vs. west coast	0.99 (0.87–1.53)		→	
	Fish consumption low (<0.5 meals/month)	1			
	Medium (1–1.5 meals/month)	East: 1.16 (0.88–1.53)			
	High (>2 meals/month)	East: 1.27 (0.96–1.69); west: 1.36 (0.96–1.94)		(↓)	
[56], USA	Mothers exposed to DDT and DDE	Fecundability ratio (95% CI)	Per 10 ng/g DDE: 16% (6–27) increase	↑	Unadjusted (potential confounders only minor effects on estimate)
		Per 10 ng/g DDT: 32% (11–48) decrease		↓	

→: no effects on TTP; ↓: significant decrease in TTP; (↓): decrease in TTP not statistically significant; ↑: significant increase in TTP (subgroup affected).

5.2. Cancer in female reproductive organs

Endogenous estrogen is a known risk factor for endometrial cancer and the relationship between endogenous estrogen and endocrine active organochlorine compounds has therefore been investigated. The two case-control studies conducted to date both indicate that at most some of the PCB and DDT congeners may cause a small, increased risk for endometrial cancer [58,59]. Relationships between organochlorine exposure and other cancers of the female reproductive organs have, to our knowledge, not been demonstrated. In the population-based studies, these rare events can only be studied with sufficient statistical power if very large populations are investigated [60].

6. Reproductive problems during pregnancy

6.1. Spontaneous abortion

About 15% of all clinically recognized pregnancies end up as spontaneous abortions. Inclusion of the very early, unrecognized pregnancies yields a proportion of spontaneous abortions close to 50% of all conceptions [61]. No increase in the frequency of spontaneous abortions was observed in the population from Taiwan that was highly exposed after consumption of contaminated cooking oil (median serum PCB level of 46 ng/g right after the exposure). However, this study included a limited number of cases: a total of 23 spontaneous abortions in 336 women were observed during a period of 14 years after the accidental exposure [41]. In India a hospital-based case-referent study reported a high organochlorine pesticide concentration (sum of BHC, Lindane, Aldrin, *p*,*p*′-DDE, *p*,*p*′-DDD, and *p*,*p*′-DDT) in the blood of women having spontaneous abortions (range from 226 to 1886 ng/g). A somewhat lower concentration was found in women giving preterm birth (145–684 ng/g) and the lowest concentration of organochlorine pesticides (34–163 ng/g) was observed in the blood from women giving birth to full-term babies [62]. Other retrospective studies of the organochlorine level in patients after spontaneous abortions indicate that organochlorines may play a role in spontaneous abortions [43,63,64]. Unfortunately, in the largest study including 120 woman hospitalized for miscarriage and 120 full-term controls [64], the organochlorine analyses were performed on pools of 10 women and the analyses were not adjusted for the measured potential confounders (age and alcohol consumption). A prospective study was set up to address the effects of DDTs on spontaneous abortions by recruiting female Chinese textile workers before they became pregnant, and by following the pregnancy outcome [61]. The odds for spontaneous abortion rose with increasing total DDT and *p*,*p*′-DDE. Unfortunately, the blood samples for DDT measurements were drawn up to 2 years after the index pregnancy, and case and control groups may have differed in terms of excretion during lactation. This was accounted for in a sensitivity analysis, which indicated that the negative association between *p*,*p*′-DDE and spontaneous abortion would persist if lactational excretion of DDE did not exceed 7% per month. However, prospective exposure assessments are needed to fully elucidate this issue. In the presumably PCB-exposed sports fishermen population in Wisconsin, or New York State, USA [65,66] and among fishermen populations from Sweden [67], no increase in the number of miscarriages was found compared with their respective control groups.

7. Reproductive problems at birth

7.1. Perinatal mortality

An elevated perinatal mortality was observed in the highly exposed Yucheng population in Taiwan [41], but none of the studies with lower exposure levels have reported an increased fetal or infant mortality (Appendix A).

7.2. Birth weight and preterm delivery

The issue of whether contamination with PCBs or DDTs causes preterm delivery and small for gestational age babies has been investigated in a number of studies. Exposure to high concentrations of PCBs and PCDFs after the Taiwanese oil poisoning caused the proportion of children born with low birth weight (<2500 g) to reach 27.7% versus 6.3% for the general population. This is due partly to a similarly high proportion of premature (<37 weeks) births (24.6% versus 8.1%) [68]. Reduced birth weight was also observed after the other large accident of oil poisoning in Japan [69], and in capacitor workers a negative relation between the birth weight of their offspring and maternal PCB exposure was found [70]; however, the findings were not as consistent in groups of the general population exposed to lower levels of these compounds (see Table 3). A large and well-designed study including American mothers giving birth between 1959 and 1966 (when DDT was still being used) found a strong dose–response relationship between DDE concentration in maternal serum on the one hand and preterm delivery, low birth weight, and growth retardation on the other hand [71]. The children in the high exposure group (>60 ng/g) weighed 150 g less and were born about one week earlier than the children in the low exposure group (<15 ng/g). The risk for preterm delivery and small for gestational age began to increase at a DDE concentration of >10 ng/g, and it applied to more than 80% of the study population. This comprehensive study used blood samples drawn when the women were pregnant (third trimester) and the analysis for DDT and metabolites several years later is therefore not expected to confer bias owing

Table 3
Epidemiological studies addressing effects of persistent organochlorines on birth weight

Reference and place	Exposure groups/compounds	Mean (median) or range of exposure	Mean, median, or midrange estimated as maternal serum PCB or DDE[a]	Birth weight
[78], Michigan, USA	Fish eaters – PCBs	2.5 ng/g PCB as Arochlor 1260 in cord serum; 5.5 ng/g in maternal serum	5.5 ng/g	↓
[70], New York State, USA	Working women exposed to PCB	9–33 ng/g Arochlor 1254 in serum, only measured on subset	21 ng/g	↓
[65], Wisconsin, USA	Fish eaters (PCBs)	0.6–5 ng/g estimated as sum of 13 congeners in maternal serum on subset	2.8 ng/g	↑
[68], Taiwan	PCB, PCDF maternal exposure	No exposure assessment	–	↓
[92], Sweden	Fishermen's wives east coast (high PCB) vs. west coast (lower PCB)	No exposure assessment	–	↓
[76], Sweden	East coast fishermen's wives high vs. low fish consumption, living in fishing village	No exposure assessment	–	↓
[79], The Netherlands	General population (PCBs)	0.80 ng/g, sum PCB-118, -138, -153, -180 in cord serum	1.6 ng/g	↓
[18], Sweden	East coast fishermen's wives CB-153 estimated in blood >300 ng/g lipid in plasma	0.9 ng/g, determined as CB-153 in plasma (lipid-adjusted 20–780 ng/g)	3.4 ng/g	↓
[80], Rural Finland	General population PCDDs + PCDF PCB	Sum of PCB 140–1624 ng/g fat in breast milk	5.9 ng/g	→
[71], USA	*p,p′*-DDE increasing concentrations	25 ng/g in serum	25 ng/g	↓
[81], Faroe Islands	Fishing community (PCB)	Sum of PCB measured in maternal serum 860 ng/g lipid	2.2 ng/g	→
[73], India	General population (*p,p′*-DDE) HCHs Other DDTs	7.6 ng/g in maternal blood	7.6 ng/g	↓ ↓ →
[75], Ukraine	General population (DDE, PCB)	DDE: 2457 ng/g milk fat Sum PCB 605 ng/g milk fat	16.4 ng/g DDE 4.0 ng/g PCB	→

→: no effects on birth weight; ↓: significant decrease in birth weight; ↑: significant increase in birth weight.
[a] Values approximated by conversions – see text for details.

to the very low decay of these compounds in frozen blood samples.

Studies performed when organochlorine pesticides were still used or in places where they are currently used report a similar relation between organochlorine exposure and preterm birth or intrauterine growth retardation [72–74]. However, the present blood levels of DDE in US and European populations are considerably lower. Recent studies hence reported median serum concentrations of *p,p′*-DDE below 10 ng/g [6]. Moreover, a recent study from Ukraine detected no association between the organochlorine concentration in breast milk and birth weight [75].

In fishermen's families living near the Baltic Sea, the birth weight was inversely related to fish consumption [76]. It was later confirmed that this was likely to be caused by PCB exposure. Blood samples were analyzed for PCB in 1995 and modeled to the time of conception up to 22 years earlier. The odds ratio (95% CI) for low birth weight (<2750 g) was 2.1 (1.0–4.7) for CB-153 >300 ng/g lipids and 2.3 (0.9–5.9) for CB-153 >400 ng/g lipids [18]. The Baltic Sea area fishermen's sisters also had an increased risk of having an infant with low birth weight (OR 1.6, 95% CI 1.1–2.3) compared with fishermen's sisters from the less polluted Swedish west coast [77]. In another fish consumer study, 240 newborn infants whose mothers consumed moderate quantities of contaminated Lake Michigan fish and 71 infants whose mothers did not eat such fish were examined during the immediate postpartum period [78]. Cord serum PCB concentration (average 2.5 ng/g) predicted lower birth weight and smaller head circumference. Furthermore, in a population-based study from the Netherlands, cord and maternal plasma PCB levels were both negatively associated with birth weight [79]. Infants with high cord plasma levels (90th percentile = 0.80 ng/g) on average weighted 165 g less than infants with low cord

plasma PCB levels (10th percentile = 0.20 ng/g). Furthermore, cord and maternal plasma PCB levels were both significantly associated with lower growth rate from birth to 3 months, but no effects of prenatal PCB exposure on growth rate were found from 3 to 42 months of age. A Finnish study reported a weak negative correlation between breast milk concentration of PCDDs/PCDFs and birth weight, but no significant correlation between PCB and birth weight [80]. A Faeroese study also found no significant correlation between PCB and birth weight [81], but a positive correlation between PCB contamination and birth weight was observed in fish consumers from the Green Bay area, Wisconsin [65]. The lack of association or positive association between PCB exposure and birth weight in some of the fish consumer studies could be due to the presence of n−3 fatty acids in fish, which has been demonstrated to have a positive effect on birth weight [82], counteracting the apparent negative effects of organochlorines.

In addition to different $n - 3$ fatty acid level, the slight inconsistencies among the studies could simply be due to different exposure levels. Using the conversion factors described in section 3, the estimated serum concentrations of PCB or DDE were calculated and included in Table 3 for comparisons among studies. It can be seen that the few studies demonstrating no effect on birth weight seem to represent exposure in the lower range, but not the lowest exposed populations.

7.3. Congenital malformations

A number of dysmorphic features at birth, including hyperpigmentation, neonatal teeth, small nails, and delay of developmental milestones, were observed in the Yucheng children exposed to high concentrations of PCB in utero [83]. These severe malformations have not been reported in populations exposed to lower concentrations of persistent organochlorines.

The malformation rates among infants born to Swedish fishermen's wives and sisters from the Baltic Sea area were somewhat lower than those of fishermen's wives and sisters from the Swedish west coast and in the same range as those of the general Swedish population, indicating that exposure to persistent organochlorines from fatty fish did not increase the risk of congenital malformations [77,84]. However, the rarity of the occurrence of specific malformations in these populations made it impossible to obtain sufficient statistical power for such analysis.

Studies of congenital malformations of reproductive organs in relation to organochlorine exposure are limited. A case-control study including 18 cryptorchidism cases and 30 controls indicated higher heptachloroepoxide and hexachlorobenzene in fat samples from cases, but not elevated levels of a range of other organochlorine compounds studied [85], and this may therefore be a chance finding. Another large case-reference study including 241 cases of cryptorchidism and 214 cases of hypospadias [86] investigated the *p,p′*-DDE level in serum samples taken during pregnancy in 1959–1966 in the USA when DDT was still being used. Mothers giving birth to sons with cryptorchidism or hypospadias had similar median serum levels of *p,p′*-DDE as mothers who gave birth to sons without malformations (median recovery adjusted serum DDE, respectively, 34.1, 34.1, and 34.3 ng/g). However, in this study a small and insignificantly increased cryptorchidism and hypospadias rate was found at *p,p′*-DDE serum level >85.6 ng/g compared with <21.4 ng/g, odds ratio (95% CI) 1.3 (0.7–2.4) for cryptorchidism and 1.2 (0.6–2.4) for hypospadias (adjusted for race, triglyceride level and cholesterol level). None of the following potential confounders affected the estimate by more than 11%: season of birth, mother's age, parity, socioeconomic index, body mass index before pregnancy, weight gain during pregnancy, hyperemensis gravidarum, hypertension, age at menarche, history of infertility, menstrual cycle irregularity, estrogen use during pregnancy, methods of delivery and serum sodium level. This study indicated that *p,p′*-DDE at most has a minor effect on the occurrence of cryptorchidism and hypospadia in countries where DDT is still being used, but the present level of *p,p′*-DDE exposure in the USA and Europe cannot explain the increasing trend of these malformations. Thus, is unlikely that exposure to organochlorines in the pre- or perinatal period are main contributors to reproductive organ malformations.

7.4. Sex ratio

A decrease in the male/female sex ratio has been reported in several, but not all countries during the past 50 years [87]. This alteration can either occur because of sex-specific embryonic mortality or because of sex chromosome specific differences in the fertilization ability of the sperm cells. Contaminant-induced alteration in the proportion of X and Y sperm cell production or maturation may also occur. The time of conception within the estrus cycle and coitus frequency have been identified as two sources of sex ratio variation in humans [88]. Since alterations in sex hormones are often found in either males or females giving birth to offspring with an altered sex ratio, it has been hypothesized that some of the changes in sex ratio are driven by altered sex hormone level around the time of conception or during gestation [89,90].

Some studies have reported that exposure to organochlorines or other compounds able to disturb normal endocrine function may give rise to alterations in the sex ratio. A female-biased sex ratio was hence observed among infants born to Swedish fishermen's wives from the Baltic Sea area compared with fishermen's wives from the less contaminated Swedish west coast [92]. A larger number of female children was also fathered by males accidentally exposed to high concentrations of PCB and PCDF

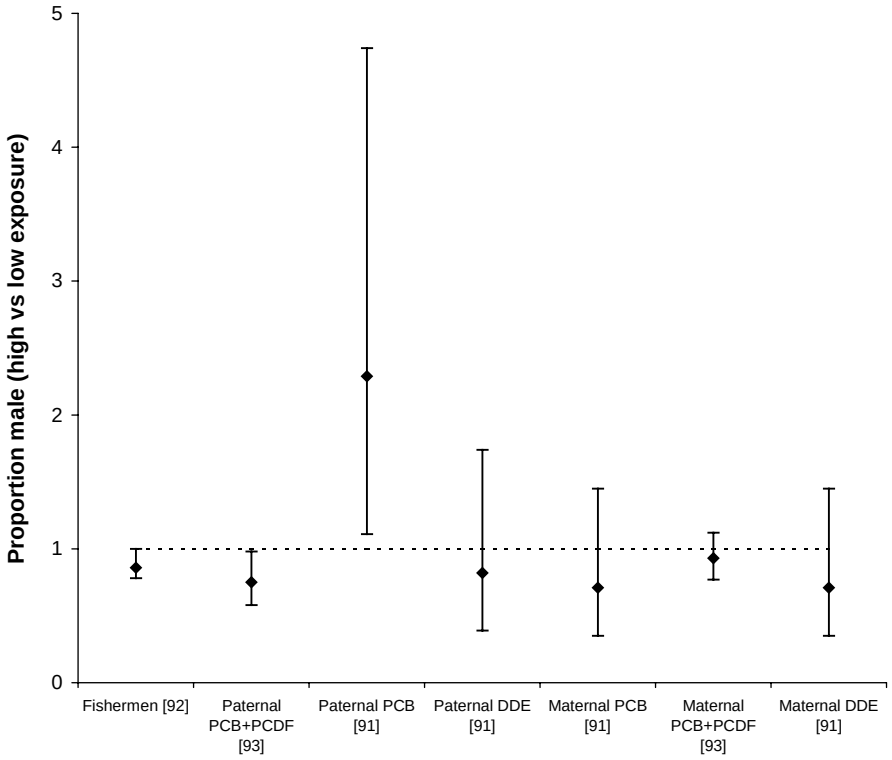

Fig. 2. Male offspring in high exposure groups in proportion to their respective control groups presented as maternal and paternal exposure to separate compounds.

[93]. On the other hand, an increased proportion of male offspring was found in one study among male fish eaters with a blood PCB concentration above 8.1 ng/g [91]. However, this study featured a small sample size compared with the other studies represented and the uncertainty of the estimation is therefore also large (Fig. 2). Maternal exposure to PCB or paternal or maternal exposure to DDE does not seem alter the sex ratio [90,92]. The potential effects of PCBs and PCB and PCDF mixtures on sex ratio may be due to the dioxin-like effects of these compounds since a marked effect on sex ratio has been observed in a study of a group of people accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Seveso, Italy. Males exposed to dioxin fathered a significantly reduced number of male offspring compared with an unexposed population, but again, female exposure did not seem to affect the sex ratio [94].

8. Development

8.1. Growth – age and size at puberty

Estrogens, androgens, and thyroid hormones are all involved in the regulation of growth and sexual maturation and the action of these hormones can be affected by several organochlorines. In humans exposed to high levels of PCBs and PCDFs (Yucheng accident), the first child born of exposed mothers had a smaller total lean mass and soft tissue mass [95], but the sexual maturation was not delayed [3]. On the other hand, a recent Dutch study indicates a negative effect of PCB on sexual maturation in boys living near incinerators [96]. Perhaps even the present level of pollution may affect growth and sexual development in part of the general population. However, some conflicting results have been reported on this subject (see Table 4). No association between PCB exposure and sexual development of boys was found in a Faeroese study [97] or of girls in an American study [98]. However, the girls (mean age 15.2 years) weighted less when the estimated prenatal PCB concentration was more than 5 ng/g (measured as Arochlor 1254, not lipid-adjusted) [99]. Exposure to DDE (a composite estimate based on breast milk, maternal blood, cord blood and placenta) was positively associated with boys' height at puberty [100] in another American study. In Belgium, the incidence of precocious puberty was 80-fold higher among adopted and non-adopted girls from developing countries than among native Belgian girls. The serum *p*,*p*′-DDE concentration (not lipid-adjusted) was also much higher in 15 adopted girls (1.2 ng/g) and 11 non-adopted foreign girls (1.0 ng/g) with precocious puberty than among the native Belgian girls with precocious puberty, where 13 out of 15 individuals had a *p*,*p*′-DDE serum concentration below detection level (0.1 ng/g). This suggests that *p*,*p*′-DDE

Table 4
Epidemiological studies addressing effects of persistent organochlorines on pubertal development

Reference	Exposure group/compound	Characters measured	Pubertal development
[98]	PCB + PBB	Age at menarche, Tanner stage	PCB → PBB ↑
[100]	PCB + DDE in mothers during pregnancy	Tanner stage	→
		Weight + height	↑
[101]	DDE	Precocious puberty	↑
[97]	PCB	Spermaturia, Tanner stage	→
[96]	PCB + dioxin	Tanner stage	↓

→: no effects on pubertal development; ↓: significantly slower pubertal development; ↑: significantly faster pubertal development.

may be one of the factors causing precocious puberty in the non-native girls [101]. Precocious puberty in foreign girls may, however, be unrelated to exposure, since this study did not control for genetic factors and other factors involved in moving from one country to another in early childhood.

9. Discussion

The epidemiological studies reviewed in this paper suggest reproductive abnormalities in human populations exposed to high concentrations of PCB or DDE, including reduced semen quality and testicular cancer in males, menstrual cycle abnormalities and spontaneous abortions in females, prolonged waiting time to pregnancy, reduced birth weight of the offspring, skewed sex ratio, and altered age of sexual development. At lower levels of exposure, such as the present level of contamination with organochlorines in the general population in Europe and North America, the reviewed studies indicate only minor, if any reproductive disturbances. However, it should be kept in mind that in the vast majority of human studies, only postnatal exposure was estimated. In the face of this stand experimental studies pointing to the fetal period as the most sensitive for the reproductive toxicity of organochlorines and other 'endocrine disrupters.'

Organochlorines are highly lipophilic and these compounds are hence mainly found in the adipose tissue, the liver and in the lipid fraction of serum and breast milk. Organochlorines can be detected in biological samples from humans from all parts of the world, but high concentrations are found especially in areas where the compounds are still being used and in humans eating large amounts of seafood at higher levels in the food chain. The serum and breast milk concentrations of PCBs and DDTs have decreased in the countries where these compounds have been banned [102,103]. However, the concentration of PCB may not decrease in people continuously exposed through contaminated seafood consumption as seen in the Michigan anglers population [104].

The mechanisms of reproductive toxicity are not known in detail, but it is most likely that the various organochlorine compounds act via somewhat different mechanisms. The compounds are relatively non-toxic when animals are acutely exposed. Using rats, an oral LD50 of 250 mg/kg was estimated for DDT and 1300 mg/kg for PCB (Arochlor 1254) (HSDB internet database). However, adverse reproductive effects such as conception delay have been observed in Rhesus monkeys exposed to down to 20 μg/kg/day Arochlor 1254 for 37 months prior to mating and through gestation [105]. The estimated average human consumption of PCB in the USA and Europe is much lower (0.005 μg/kg/day in 1982–1984 in the USA [3]). However, subpopulations eating large amounts of seafood from contaminated areas have been estimated to have a much higher intake. Among Inuits from northern Quebec, the estimated PCB intake is 0.3 μg/kg/day, and breastfed children in this population were estimated to have an even higher consumption corresponding to 10 μg/kg/day. These levels are in the range of the levels associated with delayed conception in rhesus monkeys and far exceed the EPA-established reference dose of 0.07 μg/kg/day [3].

The significance of a number of studies reporting no effects after exposure to PCBs or DDTs can be questioned. One of the major points of criticism is the lack of a proper exposure contrast in several of the studies. All humans, even people living in remote areas, have measurable concentrations of PCB and DDE in their serum, and hence a 'no exposure' group cannot be found. Correlation studies indicates that even people in the general population without a specific identifiable source of exposure may, to some extent, be affected by these compounds, but this is often only studied over a an limited exposure range [2,80]. This range may not bee large enough to allow detection of the magnitude of the possible adverse effects of the organochlorine compounds.

Furthermore, the number of included participants is often too low to detect even large differences among groups, due to the high degree of variation in most of the measured outcomes. From earlier studies, it has been estimated that detection of a difference of 33% between two groups of the same size with an 80% probability at the 5% significance level requires a minimum sample size of 150 in each group for TTP studies [106], and detection of a 25% difference between groups with the same power requires 100 in each group for sperm concentration studies [107].

Animal studies indicate that the sensitivity to organochlorine exposure is higher in fetal and neonatal than in adult life, and the transfer of these compounds to the offspring through the placental barrier during gestation and in larger amounts

via lactation therefore constitutes a particularly concerning issue [108]. Moreover, the compounds have been measured in seminal plasma, although at lower concentrations than in blood [32].

Several of the reproductive disturbances seen in humans exposed to persistent organochlorines have been corroborated in animal studies, including the effects on male and female fecundity, spontaneous abortions, reduced birth weight and altered sexual development. The effects of PCB and DDE in animal studies have recently been reviewed [3,6,13]. PCBs have been demonstrated to be reproductive toxicants in a number of species with Rhesus monkeys as the most sensitive of the tested animals. DDTs have also been demonstrated to have reproductive toxic properties at high concentrations in some, but not all animal studies, and the perinatal period is particularly vulnerable to exposure [6]. In addition, different congener compositions of the PCB and DDT mixtures result in different reproductive toxicities [3,6].

Congenital malformations of the male sexual organs, including hypospadia and chryptorchidsm, have been induced in experimental animals and wildlife exposed to DDT degradation products or other compounds with anti-androgenic properties [109]. In male rats adverse effects of PCBs on testes development and sperm cell production have only been observed in animals exposed to high concentrations, but fetal and neonatal life is associated with greater sensitivity to PCB than adult life [13]. When moderate doses of PCB are used, increases in Sertoli cell numbers and daily sperm production have been observed in male rats exposed through lactation to Arochlor 1242 (0.8 or 1.6 mg/day subcutaneously in mothers) [110]. The increased Sertoli cell numbers may be due to the concurrent decrease in thyroid hormone concentration, which has been demonstrated to limit neonatal Sertoli cell proliferation in in vitro experiments [111].

Several in vitro studies have been performed to elucidate the mechanisms of reproductive toxicology of organochlorines. A number of the PCB congeners and DDTs have been shown competent to bind to estrogen or androgen receptors and estrogenic, anti-estrogenic and anti-androgenic effects of the most common PCB congeners (PCB-138, -153, and -180) have been demonstrated [112]. Furthermore, the DDT metabolite *p,p′*-DDE exhibits anti-androgenic effects, whereas *o,p′*-DDT and *o,p′*-DDE appear to have predominantly weak estrogenic effects [6]. Although some organochlorines are present in the blood of the general population in similar or higher concentrations than the endogenous hormones, they may not cause significant disturbances of endocrine processes due to a lower affinity to the estrogen and androgen receptor of the organochlorines compared with the natural hormones [27]. However, when taking into consideration that the organochlorines have a low affinity for sex hormone-binding globulins, the bioavailable concentration of compounds with endocrine disrupting effects may be significant [113]. Furthermore, PCBs are potent inhibitors of estrogen sulphotransferase, which sulfates estradiol before urinary excreation [114]. PCBs may thus prolong the action of the natural estrogens.

All PCB congener groups have been demonstrated to be able to disrupt thyroid hormone homeostasis. Several hydroxylated PCB congeners have been demonstrated to bind with a high affinity to the thyroid transport protein transthyretin and some bind thyroid-binding globulin with a lower affinity [115]. Direct binding to the thyroid receptor has only been demonstrated for a few hydoroxylated PCBs, and these compounds bind only weakly to the receptor. This implies disruption of thyroid hormone transport as an important mechanism of the altered thyroid homeostasis in vivo [115]. Furthermore, PCBs are known to induce uridine diphosphate glucuronosyltransferase in the liver and thereby increase the excretion of thyroid hormones [116].

Although the concentration of organochlorines in the general human population is low, it cannot be precluded that these compounds may act together with other compounds to cause reproductive abnormalities. It is known from studies using the yeast estrogen receptor screen, that mixtures of compounds each at concentrations well below concentrations that have no observed effect can cause a marked endocrine response [117]. This suggests that the large number of chemicals that humans are exposed to may collectively cause reproductive problems.

Knowledge about the effects of persistent organochlorines on human reproductive health is limited and results are often conflicting. Exposure to high concentrations causes severe fetal toxicity as seen from the accidental exposure through contaminated oil in Japan and Taiwan, but the effects at lower concentrations are not as obvious and a safe level human exposure has been estimated from animal studies. Large-scale studies with a sufficient number of participants in well-defined groups with substantial exposure contrasts are needed in order to fully elucidate the possible adverse effects of persistent organochlorines on the human reproductive health. Ideally, genetic variations in the populations determining the sensitivity of an individual to gonadotoxic effects of organochlorines and other ‘endocrine disrupters’ should be identified, as such inter-individual variation may at least partly explain some of the conflicting results presented in this review. Furthermore, sufficient exposure estimates from all participants in the study is essential and these estimates should preferably be taken prospectively to avoid possible selection bias.

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Appendix A

Human studies of potentially reproductive effects of organochlorine exposure

Reference and place	Type of study	*N*	Exposure/ compound	Relevant outcome measures	Findings	Comments
Contaminated fish and seafood consumers						
[78], Michigan, USA	Case control	242 high exposed, 71 low exposed	Fish eaters, high fish consumption + PCB in cord blood	Birth weight, head circumference	Lower birth weight and head circumference in high exposed individuals	Well-controlled (37 potential confounders tested)
[65], Wisconsin, USA	Cohort	1027	Fish consumption, PCB	Fetal loss, intrauterine death, live birth, birth weight	Positive association between PCB and birth weight among women with moderate weight gain	Low exposure; PCB only measured in a subset – estimated from fish intake in others
[66], New York State, USA	Nested case control	471 cases, 1349 controls	Fish consumption, PCB exposure index	Spontaneous fetal death	Not elevated risk of spontaneous fetal death of women eating contaminated fish, risk reduction in subgroup 3 or more children	Only estimated exposure – uncertain measure, not consistent results
[92], Sweden	Cohort – register data	1501 east coast, 3553 west coast children	Swedish fishermen families	Birth weight + sex ratio	East coast fishermen families lower birth weight + lower sex ratio	No contaminant measures
[76], Sweden	Case-referent	72 cases, 162 referents	Fish consumption Swedish fishermen families east coast	Birth weight	Low birth weight associated with high fish consumption – especially for boys	No exposure measurement
[50], New York State, USA	Cohort – cross section	874	Fish consumption, PCB exposure index	TTP	No adverse effects on TTP observed in fish eaters	No exposure assessment
[42], New York State, USA	Cohort – cross section	2223	Fish consumption, PCB exposure index	Menstrual cycle length (questionnaire)	Frequent and long-term fish consumption related to shorter menstrual cycles (average 1 day shorter)	Not direct exposure assessment
[18], Sweden	Nested case control	57 cases, 135 controls	CB-153 in serum Swedish east coast, estimated at pregnancy 4–22 years back from present values	Birth weight	Increased risk of low birth weight when CB-153 >300 ng/g lipid	Indicates negative relation between *estimated* PCB and birth weight
[49], Michigan, USA	Cohort	626	Fish consumption	Conception delay (ever experienced TTP >12 months)	High paternal but not maternal fish consumption associated with increased risk of conception delay	No direct contaminant measure, not corrected for coitus frequency

Appendix A (*Continued*)

Reference and place	Type of study	*N*	Exposure/ compound	Relevant outcome measures	Findings	Comments
[48], New York State, USA	Cohort	785 couples	Fish consumption, PCB exposure index	TTP	High paternal fish consumption, no effects on TTP	No association demonstrated, only estimation of PCB contamination performed
[47], New York State, USA	Cohort	595	Fish consumption, PCB exposure index	TTP	High or long-term maternal fish consumption increases TTP	PCB only estimated from fish consumption
[67], Sweden	Cohort	443 east coast, 991 west coast	Fish consumption – PCB exposure index, CB-153 in a subset	Miscarriages + stillbirths	Not elevated risk in high exposure group	Exposure only measured on a subset, not at time of miscarriages or stillbirths
[53], Sweden	Cohort	399 east coast, 936 west coast	Fish consumption, Swedish fishermen's wives	TTP	TTP increased in smokers from east coast (eating PCB contaminated fish)	No exposure assessment, retrospective measures TTP
[81], Faroe Islands	Cohort	182	Cord serum PCB	Gestational length, birth weight, placental weight	PCB associated with fatty acids, which were associated with birth weight, no direct association birth weight PCB	Fatty acids much stronger determinators of birth weight than PCB (no effect in this study)
[55], Sweden	Cohort	121	PCB-153 in plasma Swedish east coast fishermen's wives	TTP	No relation between estimated PCB (back-calculated) and TTP	TTP did not seem to be related to PCB exposure around conception time
[51], New York State, USA	Cohort	895	Fish consumption, PCB exposure index	Fecundability	Fish consumption tended to increase risk of infecundity or subfecundity (>12 months to pregnancy)	Not significant effects, not measured contaminant
[54], Sweden	Cohort	709 east coast, 1103 west coast	Fish consumption	TTP + miscarriages	No significant association with cohort or fish consumption, high fish consumption tended to reduce risk of miscarriages + reduce TTP	No direct contaminant measure
[91], Michigan, USA	Cohort – nested	208	Fish eaters PCB + DDE in parental serum	Sex ratio	More male offspring when paternal PCB >8.1 ng/g	Wide error bars
[97], Faroe Islands	Cohort, follow-up (14 years)	196	PCB measured in umbilical cord	Spermaturia, Tanner stage, testicular volume, testosterone, LH, FSH, SHBG, Inhibin B	No relation between PCB and spermaturia as a measure of puberty, nor significant relations to sex hormones	Puberty does not seem to be affected by perinatal PCB exposure in Faroese children
Accidental exposure						
[68], Taiwan	Case-referent	130 cases compared with general population	PCB + PCDF	Mothers reproductive history, birth weight, gestation age	Premature delivery and smaller babies, but only the first years after poisoning	Suggests lactation + general elimination decreases PCB, etc. during a few years
[95], Taiwan	Matched case control	55 cases, 55 controls	PCB + PCDF	Growth, bone mineral density and soft tissue composition	Smaller, less total lean mass and soft tissue mass of first child after accident	
[34], Taiwan	Case control	Exposed 12, unexposed 23	PCB + PCDF exposure prenatal	Measured when >16 years, Tanner stage, sperm count, morphology, motility, hamster oocyte penetration	Abnormal morphology, motility and oocyte penetration capacity of sperm of boys exposed in utero	No direct measure of exposure given, low *n*, but suggests adverse effects on sperm quality after high PCB + PCDF exposure

Appendix A (*Continued*)

Reference and place	Type of study	*N*	Exposure/ compound	Relevant outcome measures	Findings	Comments
[41], Taiwan	Case control	356 cases, 312 controls	PCB + PCDF Only PCB measured in exposed group	Retrospective questionnaire info on menstrual cycle, number children, spontaneous abortion, child death, stillbirths, infertility	Abnormal menstrual bleeding, tendency to increased number of stillbirth, increased death of children, but not increased infertility and same average number of children	Increased reproductive problems in highly PCB + PCDF-exposed women
[98], Michigan, USA	Cohort	327 females, 5–24 years	PBB + PCB	Pubertal development	PBB: earlier age at menarche and earlier pubic hair development if high exposure; PCB: no significant association	PBB may advance pubertal development in girls
[93], Taiwan	Cohort	902 mothers, 469 fathers	PCB cooking oil	Sex ratio	Decreased sex ratio, especially in fathers exposed when <20 years	
[99], Michigan, USA	Cohort, follow-up	308	PCB (PBB), in serum Michigan accidental contamination 1973	Growth (height + weight)	PCB >5 ng/g = median (prenatal – estimated) reduced weight adjusted for height in daughters, 5–24 years	PCB may affect growth
[35], Taiwan	Case control	40 exposed, 28 unexposed	PCB + PCDF exposure as teenagers or adult	Sperm count, morphology, motility, hamster oocyte penetration	Increased rate of abnormal morphology and oligospermia and decreased oocyte binding and penetration	Same as above in teenagers or adults seems to induce permanent changes
Occupational exposure						
[28], Maryland, USA	Case control	38 transformer repairmen, 17 former transformer repairmen and 56 comparison workers	Transformer repairmen; PCB measured in serum and lipid	17-ketosteroids, sperm count, thyroid hormones	Thyroid hormone status different in transformer repairmen and non-repairmen – no effects on 17-ketosteroids or sperm count	No evidence for direct reproductive effects of PCB
[70], New York State, USA	Case control	172 births direct exposure + 184 births indirect exposure	Female capacitor workers; PCB	Offspring birth weight and gestational age	Birth weight and gestational age weekly correlated to PCB exposure	Exposure estimated from air concentration
General population						
[80], Rural Finland	Cross section	167	PCB + dioxins milk	Birth weight, height	Slightly decrease in birth weight, when split, only boys exposed to dioxins were affected	No clear evidence of PCB or dioxin caused birth weight decrease
[79], The Netherlands	Cross section	207 children: 105 breast fed, 102 formula fed	PCB – cord and maternal serum	Birth weight and growth up to 42 months	Lower birth weight and growth first 3 months in high PCB exposure group, no effect of breast feeding on growth	

Appendix A (*Continued*)

Reference and place	Type of study	*N*	Exposure/ compound	Relevant outcome measures	Findings	Comments
[100], North Carolina, USA	Follow-up annual questionnaires	594	PCB, DDE in mothers serum during pregnancy + cord blood	Height, weight, pubertal development	DDE increased boys height + weight at puberty, white PCB-exposed girls heavier	Prenatal exposure may effect body size at puberty
[61], China	Case control	15 cases + 15 controls	Serum DDTs, PCB female textile workers	Spontaneous abortion	DDE associated with increased risk of spontaneous abortions	Exposure measured 2 years after abortions
[71], USA	Cohort	2380 total, 361 preterm, 221 small for gestational age	DDE concentration in mothers serum during pregnancy 1959–1966	Preterm birth, small for gestation age	Strong correlation between DDE and risk of preterm birth + small for gestation age	Strongly suggest relation between DDE and preterm birth, however, not current level USA
[8], Mexico	Cross section	24	*p,p′*-DDE in serum	Sperm concentration, volume, SHBG, testosterone	*p,p′*-DDE negatively correlated to semen volume, sperm concentration and free/bound testosterone, positively correlated to SHBG	Low *n*, part of general population with high exposure, average sperm count similar to other studies (lower exposure)
[96], The Netherlands	Cross section	120 girls, 80 boys	PCBs + dioxin (Calux assay)	Pubertal development, testicular volume	Retarded sexual development in adolescents living in contaminated areas	Examined by different physicians
[86], USA	Nested case control	241 cryptochidism, 214 hypospadia, 185 polythelia, 599 controls	*p,p′*-DDE in serum, taken during pregnancy 1959–66	Cryptochidism, hypospadia, polythelia	Trend to slightly increased cryptochidism and polythelia in high exposure group – non-conclusive	Previous high level (USA) of DDE may have affected incidence of crytochidism and polythelia
[40], North Carolina, USA	Cross section	1407	*p,p′*-DDE, PCBs	Age at onset of natural menopause	Suggests later onset at natural menopause at high DDE – not significant	Breast cancer study, selection bias, organochlorine measured up to 20 years after menopause
[72], Mexico	Case control	133 cases, 100 controls	DDE, HCH, and HCB in maternal serum	Preterm birth	Suggests increased risk of preterm birth at high DDE and HCH level	Wide confidence intervals – not statistically significant
[73], India	Case control	30 cases, 24 controls	DDTs, HCH in maternal blood, placenta, and cord blood	Intrauterine growth retardation, birth weight	Association between maternal blood level of DDTs, HCH, and intrauterine growth retardation and birth weight	Results from present day high use area
[75], Ukraine	Cross section	197	DDT, DDE, PCBs, HCH, HCB in breast milk	Birth weight	No relation after adjustment for potential confounders	
[2], Sweden	Cross section	305	CB-153	Testis size, sperm concentration, motility, serum level of FSH, LH, Inhibin B, testosterone, SHBG and estradiol	Testosterone/SHBG + sperm cell motility negatively correlated to CB-153, other outcomes unaffected	Low exposure group, correlations not very strong, $r < 0.26$
[56], California, USA	Cohort	289	DDT, DDE in maternal serum	Daughters time to pregnancy	DDT increased TTP; DDE decreased TTP	
Hospital-based case-referent studies						
[63], Israel	22 women, 15 missed abortion cases	Organochlorine insecticides + PCB in blood	Missed abortions	46% of missed abortion cases had increased PCB level	Low *n*, no statistics but clear indications	

Appendix A (*Continued*)

Reference and place	Type of study	*N*	Exposure/ compound	Relevant outcome measures	Findings	Comments
[62], India	15 preterm labor, 10 spontaneous abortion, 25 control	BHC, Lindane, Aldrin, *p*,*p*′-DDE, *p*,*p*′-DDD, *p*,*p*′-DDT, in maternal serum + placenta + fetus	Spontaneous abortion, preterm birth	General organochlorine level: spontaneous abortion > preterm > control	High exposure population, seven-fold difference between mean OC in spontaneous abortion and control group – low *n*	
[74], Israel	17 premature delivery cases, 10 control	PCBs, DDTs, Lindane, dieldrin, hepatochlor epoxide	Premature delivery	All organochlorines higher in cases, suggests association	Low *n*	
[29], New York State, USA	170 patients	PCB + DDE in seminal plasma	Sperm count and motility	Among patients <20 million/ml negative correlation between 3 PCB congeners and motility	Initial study – indicates trends – non-conclusive	
[64], Italy	120 miscarriage cases, 120 control (in pools of 10 – effective $n = 12$ in each group)	PCB (Fenchlor 54), DCB, HCB, DDEtot, DDTtot, BHCs	Miscarriage	Reports higher PCB (Fenchlor 54) in cases, age and alcohol positively correlated, whereas milk and fish consumption negatively correlated to PCBs	Results flawed by using unpooled sample size in analysis, not adjusted for measured confounders	
[33], Germany	174 ejaculates, 154 patients	PCB, etc. in seminal plasma	Semen volume, sperm concentration, motility and morphology	Normal sperm highest PCB	No effect on individual measures	
[44], Canada	86 endometriosis cases, 70 control	PCBs, chlorinated pesticides	Endometriosis	No indications of association between PCB or chlorinated pesticides and endometriosis	Low exposure in general population does not seem to effect endometriosis	
[58], USA	90 cases, 90 controls	PCBs, DDTs in seminal plasma	Endometrial cancer + potential confounders	Organochlorine exposure did not seem to be related to endometrial cancer		
[43], Germany	489 women with gynecological disorders	OCs including PCB and DDE	Correlations between gynecological disorders and OCs	Several gynecological disorders seemed to be related to OCs but age only significant factor in multivariate analysis	Indicates reproductive effects of OCs, not conclusive	
[59], Sweden	154 cases, 205 controls	DDTs and PCBs in serum	Endometrial cancer + potential confounders	Organochlorine exposure did not seem to be related to endometrial cancer		
[85], Germany	18 cases, 30 controls	DDTs, PCBs toxaphenes, etc.	Undescended testis	Hexachlorobencene (HCB) and heptachloroepoxide (HCE) related to undescended testis	Low *n*, explorative study, no effects of PCB or DDE indicated	
[101], Belgium	26 foreign girls, 15 Belgium girls	DDE	Precocious puberty	Ten-fold higher DDE concentration among foreign girls with precocious puberty	Low *n*, not controlled for genetic differences and environmental differences caused by moving to another country	
[45], Belgium	42 endometriosis cases, 27 controls	PCB + dioxin like activity (Calux assay)	Endometriosis	No significant association between PCB or dioxin like activity and endometriosis detected	Slightly higher level in cases – larger sample size needed to draw firm conclusions	

Appendix A (*Continued*)

Reference and place	Type of study	*N*	Exposure/ compound	Relevant outcome measures	Findings	Comments
[31], MA, USA	29	PCB, DDE in serum – normal subjects	Semen volume, sperm concentration, motility and morphology	Trend of association between PCB + DDE and semen motility, quantity and morphology	Generally higher concentration of organochlorines in undescended testis group	
[32], India	21 infertility cases (unknown factor), 32 controls	PCB, phthalate esters	Semen volume, sperm concentration, motility, and morphology, vitality, osmoregulatory capacity, sperm chromatin structure, and Sperm nuclear DNA integrity	PCBs only detectable in seminal plasma of cases, ejaculate volume, progressively motile sperm, and vitality negatively correlated to PCB	Low *n*, for some PCB congeners extremely high detection levels, which cast doubt on the analytical quality	
[30], The Netherlands	65 persons, 31 with normal sperm counts	PCBs + metabolites + DDTs in fertility treatment patients blood + seminal plasma	Semen volume, sperm concentration, motility and morphology	PCB metabolite negatively related to sperm count and progressive motility among men with normal sperm count	More significant correlations – emphasis on the ones that fits the theory	
[57], Sweden	61 cases, 58 controls	PCB, *p*,*p*′-DDE, HCB and chlordanes in serum of case + control + their mothers	Testicular cancer	Significant increased odds of testicular cancer related to: case: *cis*-nonachlordane; case mothers: PCB, HCB, *trans*- and *cis*-nonachlordane	Suggests effects of exposure in utero, but to a lesser degree postnatal	
[72], Mexico	133 cases, 100 controls	DDE, HCH, and HCB in maternal serum	Preterm birth	Suggests increased risk of preterm birth at high DDE and HCH level	Wide confidence intervals – not statistically significant	
[73], India	30 cases, 24 controls	DDTs, HCH in maternal blood, placenta, and cord blood	Intrauterine growth retardation, birth weight	Association between maternal blood level of DDTs, HCH and intrauterine growth retardation and birth weight	Results from present day high use area	

References

[1] Longnecker MP, Rogan WJ, Lucier G. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health. Annu Rev Public Health 1997;18:211–44.

[2] Richthoff J, Rylander L, Jönsson BAG, Åkesson H, Hagmar L, Nilsson-Ehle P, et al. Serum level of 2,2′,4,4′,5,5′-hexachlorbiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. Environ Health Perspect 2003;203:409–13.

[3] ATSDR. In: Faroon OM, Olson J, editors. Toxicological profile for polychlorinated biphenyls; 2000.

[4] Breivik K, Sweetman A, Pacyna JM, Jones KC. Towards a global historical emission inventory for selected PCB congeners—a mass balance approach. 1. Global production and consumption. Sci Total Environ 2002;290:181–98.

[5] Katami T, Yasuhara A, Okuda T, Shibamoto T. Formation of PCDDs, PCDFs, and coplanar PCBs from polyvinyl chloride during combustion in an incinerator. Environ Sci Technol 2002;36:1320–4.

[6] ATSDR. In: Faroon OM, Harris OM, Llados F, Swarts S, Sage G, Citra M, Gefell, D, editors. Toxicological profile for DDT, DDE and DDD; 2002.

[7] Smith D. Worldwide trends in DDT levels in human breast milk. Int J Epidemiol 1999;28:179–88.

[8] Ayotte P, Giroux S, Dewailly E, Hernandez AM, Farias P, Danis R, et al. DDT spraying for malaria control and reproductive function in Mexican men. Epidemiology 2001;12:366–7.

[9] Toppari J, Skakkebaek NE. Sexual differentiation and environmental endocrine disrupters. Baillieres Clin Endocrinol Metab 1998;12:143–56.

[10] Lione A. Polychlorinated biphenyls and reproduction. Reprod Toxicol 1988;2:83–9.

[11] Foster WG. The reproductive toxicology of Great Lakes contaminants. Environ Health Perspect 1995;103:63–9.

[12] Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. Crit Rev Toxicol 1995;25:133–63.

[13] Faroon OM, Keith S, Jones D, De Rosa C. Effects of polychlorinated biphenyls on development and reproduction. Toxicol Ind Health 2001;17:63–93.

[14] Schantz SL, Widholm JJ, Rice DC. Effects of PCB exposure on neuropsychological function in children. Environ Health Perspect 2003;111:357–576.

[15] Snedeker SM. Pesticides and breast cancer risk: a review of DDT, DDE, and dieldrin. Environ Health Perspect 2001;109(Suppl 1):35–47.

[16] Moysich KB, Menezes RJ, Baker JA, Falkner KL. Environmental exposure to polychlorinated biphenyls and breast cancer risk. Rev Environ Health 2002;17:263–77.

[17] Hagmar L, Rylander L, Dyremark E, Klasson-Webler E, Erfurth EM. Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. Int Arch Occup Environ Health 2001;74:184–8.

[18] 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;147:493–502.

[19] Auger J, Kunstmann JM, Czysglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med 1995;332:281–5.

[20] Irvine DS. Falling sperm quality. BMJ 1994;309:476.

[21] Van Waeleghem K, De Clercq N, Vermeulen L, Schoonjans F, Comhaire F. Deterioration of sperm quality in young healthy Belgian men. Hum Reprod 1996;11:325–9.

[22] Bonde JP, Kold JT, Brixen LS, Abell A, Scheike T, Hjollund NH, et al. Year of birth and sperm count in 10 Danish occupational studies. Scand J Work Environ Health 1998;24:407–13.

[23] Andersen AG, Jensen TK, Carlsen E, Jorgensen N, Andersson AM, Krarup T, et al. High frequency of sub-optimal semen quality in an unselected population of young men. Hum Reprod 2000;15:366–72.

[24] Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 1998;352:1172–7.

[25] Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette Jr LJ, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 1996;104(Suppl 4):741–803.

[26] Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 2001;16:972–8.

[27] Safe SH. Endocrine disruptors and human health–is there a problem? An update. Environ Health Perspect 2000;108:487–93.

[28] Emmett EA, Maroni M, Jefferys J, Schmith J, Levin BK, Alvares A. Studies of transformer repair workers exposed to PCBs: II. Results of clinical laboratory investigations. Am J Ind Med 1988;14:47–62.

[29] Bush B, Bennett AH, Snow JT. Polychlorobiphenyl congeners, *p,p′*-DDE, and sperm function in humans. Arch Environ Contam Toxicol 1986;15:333–41.

[30] Dallinga JW, Moonen EJC, Dumoulin JCM, Evers JLH, Geraedts JPM, Kleinjans JCS. Decreased human semen quality and organochlorine compounds in blood. Hum Reprod 2002;17:1973–9.

[31] Hauser R, Altshul L, Chen ZY, Ryan L, Overstreet J, Schiff I, et al. Environmental organochlorines and semen quality: results of a pilot study. Environ Health Perspect 2002;110:229–33.

[32] Rozati R, Reddy PP, Reddanna P, Mujtaba R. Role of environmental estrogens in the deterioration of male factor fertility. Fertil Steril 2002;78:1187–94.

[33] Ensslen SC, Riedel H-H, Blüthgen H, Heeschen W. Chlorkohlenwasserstoff in seminalplasma und männliche Fertilität. Zentralblatt für Gynäkologie 1990;112:817–21.

[34] Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. Lancet 2000;356:1240–1.

[35] Hsu PC, Huang W, Yao WJ, Wu MH, Guo YL, Lambert GH. Sperm changes in men exposed to polychlorinated biphenyls and dibenzofurans. JAMA 2003;289:2943–4.

[36] Wijchman JG, de Wolf BT, Graafe R, Arts EG. Variation in semen parameters derived from computer-aided semen analysis, within donors and between donors. J Androl 2001;22:773–80.

[37] Appell RA, Evans PR. The effect of temperature on sperm motility and viability. Fertil Steril 1977;28:1329–32.

[38] Sundby J, Guttormsen G. Ufrivillig barnløshed. Copenhagen: Hans Reitzels Forlag; 1991.

[39] Mattison DR. In: Dixon RL, editor. Reproductive toxicology. New York: Raven Press; 1985. p. 109–30.

[40] Cooper GS, Savitz DA, Millikan R, Chiu KT. Organochlorine exposure and age at natural menopause. Epidemiology 2002;13:729–33.

[41] Yu ML, Guo YLL, Hsu CC, Rogan WJ. Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long-term follow-up interviews of the women from the Taiwan Yucheng cohort. Int J Epidemiol 2000;29:672–7.

[42] Mendola P, Buck GM, Sever LE, Zielezny M, Vena JE. Consumption of PCB-contaminated freshwater fish and shortened menstrual cycle length. Am J Epidemiol 1997;146:955–60.

[43] Gerhard I, Monga B, Krahe J, Runnebaum B. Chlorinated hydrocarbons in infertile women. Environ Res 1999;80:299–310.

[44] Lebel G, Dodin S, Ayotte P, Marcoux S, Ferron LA, Dewailly E. Organochlorine exposure and the risk of endometriosis. Fertil Steril 1998;69:221–8.

[45] Pauwels A, Schepens PJC, D'Hooghe T, Delbeke L, Dhont M, Brouwer A, et al. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-control study of infertile women. Hum Reprod 2001;16:2050–5.

[46] Olsen J. Design options and sources of bias in time-to-pregnancy studies. Scand J Work Environ Health 1999;25(Suppl 1):5–7.
[47] Buck GM, Vena JE, Schisterman EF, Dmochowski J, Mendola P, Sever LE, et al. Parental consumption of contaminated sport fish from Lake Ontario and predicted fecundability. Epidemiology 2000;11:388–93.
[48] Buck GM, Mendola P, Vena JE, Sever LE, Kostyniak P, Greizerstein H, et al. Paternal Lake Ontario fish consumption and risk of conception delay, New York State angler cohort. Environ Res 1999;80:S13–8.
[49] Courval JM, DeHoog JV, Stein AD, Tay EM, He JP, Humphrey HEB, et al. Sport-caught fish consumption and conception delay in licensed Michigan anglers. Environ Res 1999;80:S183–8.
[50] Buck GM, Sever LE, Mendola P, Zielezny M, Vena JE. Consumption of contaminated sport fish from Lake Ontario and time-to-pregnancy – New York State Angler Cohort. Am J Epidemiol 1997;146:949–54.
[51] McGuinness BM, Buck GM, Mendola P, Sever LE, Vena JE. Infecundity and consumption of polychlorinated biphenyl-contaminated fish. Arch Environ Health 2001;56:250–3.
[52] Baird DD, Weinberg CR, Schwingl P, Wilcox AJ. Selection bias associated with contraceptive practice in time-to-pregnancy studies. Ann NY Acad Sci 1994;709:156–64.
[53] Axmon A, Rylander L, Stromberg U, Hagmar L. Time to pregnancy and infertility among women with a high intake of fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 2000;26:199–206.
[54] Axmon A, Rylander L, Stromberg U, Hagmar L. Female fertility in relation to the consumption of fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 2002;28:124–32.
[55] Axmon A, Rylander L, Stromberg U, Dyremark E, Hagmar L. Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to time to pregnancy. J Toxicol Environ Health A 2001;64:485–98.
[56] Cohn BA, Cirillo PM, Wolff MS, Schwingl PJ, Cohen RD, Sholtz RI, et al. DDT and DDE exposure in mothers and time to pregnancy in daughters. Lancet 2003;361:2205–6.
[57] Hardell L, Bavel BV, Lindström G, Carlberg M, Dreifaldt AC, Wijkström H, et al. Increased concentrations of polychlorinated biphenyls, hexachlorbenzene, and chlordanes in mothers of men with testicular cancer. Environ Health Perspect 2003;111:930–4.
[58] Sturgeon SR, Brock JW, Potischman N, Needham LL, Rothman N, Brinton LA, et al. Serum concentrations of organochlorine compounds and endometrial cancer risk (United States). Cancer Causes Control 1998;9:417–24.
[59] Weiderpass E, Adami HO, Baron JA, Wicklund-Glynn A, Aune M, Atuma S, et al. Organochlorines and endometrial cancer risk. Cancer Epidemiol Biomarker Prev 2000;9:487–93.
[60] Rylander L, Hagmar L. Mortality and cancer incidence among women with a high consumption of fatty fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 1995;21:419–26.
[61] Korrick SA, Chen C, Damokosh AI, Ni J, Liu X, Cho SI, et al. Association of DDT with spontaneous abortion: a case-control study. Ann Epidemiol 2001;11:491–6.
[62] Saxena MC, Siddiqui MK, Seth TD, Krishna Murti CR, Bhargava AK, Kutty D. Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature of full-term delivery. J Anal Toxicol 1981;5:6–9.
[63] Bercovici B, Wassermann M, Wassermann D, Cucos S, Ron M. Missed abortions and some organochlorine compounds. Acta Med Leg 1980;30:177–85.
[64] Leoni V, Fabiani L, Marinelli G, Puccetti G, Tarsitani GF, De Carolis A, et al. PCB and other organochlorine compounds in blood of women with or without miscarriage: a hypothesis of correlation. Ecotoxicol Environ Saf 1989;17:1–11.
[65] Dar E, Kanarek MS, Anderson HA, Sonzogni WC. Fish consumption and reproductive outcomes in Green-Bay. Wisconsin Environ Res 1992;59:189–201.
[66] Mendola P, Buck GM, Vena JE, Zielezny M, Sever LE. Consumption of PCB-contaminated sport fish and risk of spontaneous fetal death. Environ Health Perspect 1995;103:498–502.
[67] Axmon A, Rylander L, Stromberg U, Hagmar L. Miscarriages and stillbirths in women with a high intake of fish contaminated with persistent organochlorine compounds. Int Arch Occup Environ Health 2000;73:204–8.
[68] Yen YY, Lan SJ, Yang CY, Wang HH, Chen CN, Hsieh CC. Follow-up-study of intrauterine growth of transplacental yu-cheng babies in Taiwan. Bull Environ Contam Toxicol 1994;53:633–41.
[69] Yamashita F, Hayashi M. Fetal PCB syndrome: clinical features, intrauterine growth retardation and possible alteration in calcium metabolism. Environ Health Perspect 1985;59:41–5.
[70] Taylor PR, Stelma JM, Lawrence CE. The relation of polychlorinated biphenyls to birth weight and gestational age in the offspring of occupationally exposed mothers. Am J Epidemiol 1989;129:395–406.
[71] Longnecker MP, Klebanoff MA, Zhou HB, Brock JW. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 2001;358:110–4.
[72] Torres-Arreola L, Berkowitz G, Torres-Sanchez L, Lopez-Cervantes M, Cebrian ME, Uribe M, et al. Preterm birth in relation to maternal organochlorine serum levels. Ann Epidemiol 2003;13:158–62.
[73] Siddiqui MK, Srivastava S, Srivastava SP, Mehrotra PK, Mathur N, Tandon I. Persistent chlorinated pesticides and intra-uterine foetal growth retardation: a possible association. Int Arch Occup Environ Health 2003;76:75–80.
[74] Wassermann M, Ron M, Bercovici B, Wassermann D, Cucos S, Pines A. Premature delivery and organochlorine compounds: polychlorinated biphenyls and some organochlorine insecticides. Environ Res 1982;28:106–12.
[75] Gladen BC, Shkiryak-Nyzhnyk ZA, Chyslovska N, Zadorozhnaja TD, Little RE. Persistent organochlorine compounds and birth weight. Ann Epidemiol 2003;13:151–7.
[76] Rylander L, Stromberg U, Hagmar L. Dietary intake of fish contaminated with persistent organochlorine compounds in relation to low birth weight. Scand J Work Environ Health 1996;22:260–6.
[77] Rylander L, Stromberg U, Hagmar L. Lowered birth weight among infants born to women with a high intake of fish contaminated with persistent organochlorine compounds. Chemosphere 2000;40:1255–62.
[78] Fein GG, Jacobson JL, Jacobson SW, Schwartz PM, Dowler JK. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. J Pediatr 1984;105:315–20.
[79] Patandin S, Koopman-Esseboom C, de Ridder MA, Weisglas-Kuperus N, Sauer PJ. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatr Res 1998;44:538–45.
[80] Vartiainen T, Jaakkola JJK, Saarikoski S, Tuomisto J. Birth weight and sex of children and the correlation to the body burden of PCDDs/PCDFs and PCBs of the mother. Environ Health Perspect 1998;106:61–6.
[81] Grandjean P, Bjerve KS, Weihe P, Steuerwald U. Birth weight in a fishing community: significance of essential fatty acids and marine food contaminants. Int J Epidemiol 2001;30:1272–8.
[82] Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS, et al. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet 1992;339:1003–7.
[83] Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, et al. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 1988;241:334–6.
[84] Rylander L, Hagmar L. No evidence for congenital malformations or prenatal death in infants born to women with a high dietary

intake of fish contaminated with persistent organochlorines. Int Arch Occup Environ Health 1999;72:121–4.
[85] Hosie S, Loff S, Witt K, Niessen K, Waag KL. Is there a correlation between organochlorine compounds and undescended testes? Eur J Pediatr Surg 2000;10:304–9.
[86] Longnecker MP, Klebanoff MA, Brock JW, Zhou H, Gray KA, Needham LL, et al. Maternal serum level of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. Am J Epidemiol 2002;155:313–22.
[87] Parazzini F, La Vecchia C, Levi F, Franceschi S. Trends in male:female ratio among newborn infants in 29 countries from five continents. Hum Reprod 1998;13:1394–6.
[88] James WH. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. J Theor Biol 1996;180:271–86.
[89] Krackow S. Potential mechanisms for sex ratio adjustment in mammals and birds. Biol Rev Camb Philos Soc 1995;70:225–41.
[90] James WH. The data sources which may help strengthen the epidemiological evidence for the hormonal hypothesis of sex determination in man. Hum Reprod 2001;16:1081–5.
[91] Karmaus W, Huang SY, Cameron L. Parental concentration of dichlorodiphenyl dichloroethene and polychlorinated biphenyls in Michigan fish eaters and sex ratio in offspring. J Occup Environ Med 2002;44:8–13.
[92] Rylander L, Stromberg U, Hagmar L. Decreased birth-weight among infants born to women with a high dietary-intake of fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 1995;21:368–75.
[93] Del Rio Gomez I, Marshall T, Tsai P, Shao YS, Guo YL. Number of boys born to men exposed to polychlorinated biphenyls. Lancet 2002;360:143–4.
[94] Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG, Kieszak SM, Brambilla P, et al. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 2000;355:1858–63.
[95] Guo YL, Lin CJ, Yao WJ, Ryan JJ, Hsu CC. Musculoskeletal changes in children prenatally exposed to polychlorinated-biphenyls and related-compounds (yu-cheng children). J Toxicol Environ Health 1994;41:83–93.
[96] Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect 2002;110:771–6.
[97] Mol NM, Sorensen N, Weihe P, Andersson AM, Jorgensen N, Skakkebaek NE, et al. Spermaturia and serum hormone concentrations at the age of puberty in boys prenatally exposed to polychlorinated biphenyls. Eur J Endocrinol 2002;146:357–63.
[98] Blanck HM, Marcus M, Tolbert PE, Rubin C, Henderson AK, Hertzberg VS, et al. Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. Epidemiology 2000;11:641–7.
[99] Blanck HM, Marcus M, Rubin C, Tolbert PE, Hertzberg VS, Henderson AK, et al. Growth in girls exposed in utero and postnatally to polybrominated biphenyls and polychlorinated biphenyls. Epidemiology 2002;13:205–10.
[100] Gladen BC, Ragan NB, Rogan WJ. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr 2000;136:490–6.
[101] Krstevska-Konstantinova M, Charlier C, Craen M, Du CM, Heinrichs C, de Beaufort C, et al. Sexual precocity after immigration from developing countries to Belgium: evidence of previous exposure to organochlorine pesticides. Hum Reprod 2001;16:1020–6.
[102] Schade G, Heinzow B. Organochlorine pesticides and polychlorinated biphenyls in human milk of mothers living in northern Germany: current extent of contamination, time trend from 1986 to 1997 and factors that influence the levels of contamination. Sci Total Environ 1998;215:31–9.
[103] Noren K, Meironyte D. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. Chemosphere 2000;40:1111–23.
[104] He JP, Stein AD, Humphrey HE, Paneth N, Courval JM. Time trends in sport-caught Great Lakes fish consumption and serum polychlorinated biphenyl levels among Michigan Anglers, 1973–1993. Environ Sci Technol 2001;35:435–40.
[105] Arnold DL, Bryce F, McGuire PF, Stapley R, Tanner JR, Wrenshall E, et al. Toxicological consequences of aroclor 1254 ingestion by female rhesus (*Macaca mulatta*) monkeys. Part 2. Reproduction and infant findings. Food Chem Toxicol 1995;33:457–74.
[106] Baird DD, Wilcox AJ, Weinberg CR. Use of time to pregnancy to study environmental exposures. Am J Epidemiol 1986;124:470–80.
[107] Bonde JP, Giwercman A, Ernst E. Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design options. Occup Environ Med 1996;53:511–9.
[108] Chen CY, Hamm JT, Hass JR, Birnbaum LS. Disposition of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and non-ortho polychlorinated biphenyls in pregnant Long–Evans rats and the transfer to offspring. Toxicol Appl Pharmacol 2001;173:65–88.
[109] Gray Jr LE. Xenoendocrine disrupters: laboratory studies on male reproductive effects. Toxicol Lett 1998;102–103:331–5.
[110] Kim IS. Effects of exposure of lactating female rats to polychlorinated biphenyls (Pcbs) on testis weight, sperm production and sertoli cell numbers in the adult male offspring. J Vet Med Sci 2001;63:5–9.
[111] Cooke PS, Zhao YD, Bunick D. Triiodothyronine inhibits proliferation and stimulates differentiation of cultured neonatal Sertoli cells: possible mechanism for increased adult testis weight and sperm production induced by neonatal goitrogen treatment. Biol Reprod 1994;51:1000–5.
[112] Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TH, Vinggaard AM. Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 2001;158:141–53.
[113] Hodgert JH, Zacharewski TR, Hammond GL. Interactions between human plasma sex hormone-binding globulin and xenobiotic ligands. J Steroid Biochem Mol Biol 2000;75:167–76.
[114] Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, et al. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 2000;141:1897–900.
[115] Cheek AO, Kow K, Chen J, McLachlan JA. Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. Environ Health Perspect 1999;107:273–8.
[116] Vansell NR, Klaassen CD. Increase in rat liver UDP-glucuronosyltransferase mRNA by microsomal enzyme inducers that enhance thyroid hormone glucuronidation. Drug Metab Dispos 2002;30:240–6.
[117] Rajapakse N, Silva E, Kortenkamp A. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ Health Perspect 2002;110:917–21.