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INVITED REVIEW

Reproductive Health

Persistent organic pollutants and male reproductive health

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Environmental contaminants such as persistent organic pollutants (POPs) are man-made bioaccumulative compounds with long half-lives that are found throughout the world as a result of heavy use in a variety of consumer products during the twentieth century. Wildlife and animal studies have long suggested adverse effects of exposure to these compounds on human reproductive health, which, according to the endocrine disrupter hypothesis, are ascribed to the compounds' potential to interfere with endocrine signaling, especially when exposure occurs during certain phases of fetal and childhood development. An extensive number of epidemiological studies have addressed the possible effects of exposure to POPs on male reproductive health, but the results are conflicting. Thus far, most studies have focused on investigating exposure and the different reproductive health outcomes during adulthood. Some studies have addressed the potential harmful effects of fetal exposure with respect to malformations at birth and/or reproductive development, whereas only a few studies have been able to evaluate whether intrauterine exposure to POPs has long-term consequences for male reproductive health with measurable effects on semen quality markers and reproductive hormone levels in adulthood. Humans are not exposed to a single compound at a time, but rather, to a variety of different substances with potential divergent hormonal effects. Hence, how to best analyze epidemiological data on combined exposures remains a significant challenge. This review on POPs will focus on current knowledge regarding the potential effects of exposure to POPs during fetal and childhood life and during adulthood on male reproductive health, including a critical revision of the endocrine disruption hypothesis, a comment on pubertal development as part of reproductive development and a comment on how to account for combined exposures in epidemiological research.

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INTRODUCTION

The possible effects of exposure to environmental contaminants on male reproductive function have been addressed in a number of epidemiological studies.[1–3] Although these studies suggest some effects on male reproductive health, the results across studies are often conflicting.

There are considerable numbers of animal studies that indicate adverse effects of environmental toxicants on male reproductive health in both experimental and wildlife settings,[4–6] but it should be noted that the exposure levels causing reproductive toxicity in most of these studies were several times higher than the human exposure levels measured in the general population.

Special attention has been given to the potential adverse effects on human reproductive function of compounds that are suspected to interfere with the endogenous regulation of hormonal action and thus are suspected to interfere with the development and function of reproductive hormone-regulated processes such as genital development, puberty onset and sperm production. Due to interference with hormone receptors or steroid-producing or degrading enzymes, even low concentrations of these compounds may disturb endocrine-regulated processes.[7]

Several of these compounds are suspected to affect male reproductive health. The major groups include phthalates and bisphenol A, which have typically been used in the plastic industry, polychlorinated biphenyls (PCBs), which were used extensively in transformers, insulating condensers, as plasticizers, insulators, paints and flame retardants[8] and organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB).

In recent years, other halogenated organic compounds have received increased attention, including brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and the perfluorinated compounds (PFCs) that have been used as surfactants in industrial processes and extensively as oil and water repellents for consumer products.[9] In addition to the organic compounds, certain metals (e.g. mercury, lead and cadmium) have been suspected to interfere with male reproductive health, as effects on male reproductive function have been demonstrated at high exposure levels in occupational and experimental settings.[10–12]

The biopersistent compounds, including the halogenated organic compounds, have been a subject of particular concern, as they often have half-lives in the human body of more than 5 years and are

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bioaccumulative. Several of these compounds can be detected in human serum around the globe, and a large number of these persistent organic pollutants (POPs) have been regulated to avoid further bioaccumulation in humans.

Several methods have been developed to study male fertility. Semen quality is often used as a marker of male fertility, and the probability of achieving pregnancy (fecundability) has been demonstrated to be highly dependent on concentrations of sperm of up to approximately 40 million ml^{-1}.[13–15] In addition to sperm concentration, sperm morphology and motility are part of the standard evaluation of semen quality and have also been shown to be associated with fecundability independently of sperm concentration.[13] Other attempts have been made to develop alternative measures of semen quality, including the development of different assays for the assessment of sperm chromatin integrity. Such measures have also been demonstrated to be associated with fecundability independently of standard sperm parameters.[16]

Malformations of the male genital organs (cryptorchidism and hypospadias) have been studied as markers of male reproductive disturbances, as these outcomes can usually be detected at birth. Although they can be surgically corrected, these malformations are related to reduced male fertility in adulthood, especially if left untreated.[17,18] In addition, male anogenital distance has recently been demonstrated to be related to semen quality and chance of fatherhood[19,20] and may be used as a marker of male fecundability. Furthermore, the endocrine-regulated timing of puberty has been suggested to be affected by environmental contaminants, and in addition to psychosocial consequences, early puberty onset in boys has been related to the subsequent risk of testicular cancer.[21] Finally, reproductive hormone levels are considered markers for male reproductive function.[22]

Although the majority of previous studies have addressed adult exposure and reproductive health, theoretical considerations supported by animal studies and a number of recent epidemiological studies suggest that exposure during the fetal period may have more severe long-term consequences for male reproductive health than exposure in adulthood, where the observed effects are more likely to be reversible.[2]

The present review will focus on environmental contaminants and male reproductive health, with special attention given to POPs.

PERSISTENT ORGANIC POLLUTANTS AND MALE REPRODUCTIVE HEALTH

Human exposure characterization

Generally, human exposure to POPs has declined because the production of most POPs has ceased or been significantly reduced. However, some of the more recently developed POPs are still in use or have been phased out only recently.

The majority of nonoccupationally exposed general populations are exposed to POPs through dietary intake of environmentally contaminated food, but exposure may also result from drinking water, air or dermal contact with POPs. Additionally, children are exposed to maternal POP levels during intrauterine development and postnatally through breast feeding and inhalation of dust while crawling. Moreover, for PFCs, which unlike the majority of other POPs accumulate in the blood and liver instead of adipose tissue, indirect exposure as a result of biotransformation/degradation of residual or commercial fluorochemical starting materials is also thought to be a significant source of exposure.[23]

POPs enter the environment from a number of industrial sources, including industrial waste water, incinerating plants, power stations and landfills and they are not removed by conventional waste water treatment processes. POPs can be found in water, soil and sediment, and are transported across the globe through the atmosphere and by fresh and marine water currents far from their original production or usage sites.

PCB-138, -153 and -180 are the most common PCB congeners in biological samples, and 2,2',4,4',5,5'-hexachlorobiphenyl(CB-153) is often used as a proxy for total PCB exposure because ∑PCB and CB-153 are highly correlated.[24,25] Many studies have suggested that fish consumption is a strong predictor for higher PCB concentrations, but the intake of dairy products and meat has also been associated with higher levels of PCBs.[26]

Studies on Scandinavian background exposure levels of CB-153 performed in the 2000s demonstrated this level to be in the range of 50–68 ng g^{-1} lipid,[27,28] which is similar to mean levels in the USA during the same time period (44 ng g^{-1} lipid).[29] Temporal trends in human sera suggest that the levels of perfluorinated compounds such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) peaked around the year 2000 and are currently declining.[23,30] Generally, PFC exposure is high in industrialized countries, but it is also high in the Arctic due to bioaccumulation in the food chain. Recent mean serum levels of PFOA and PFOS, as measured in Red Cross blood donors in 2010 in the USA, were 2.4 and 8.3 ng ml^{-1}, respectively, and between 2001 and 2010, these levels declined 48% for PFOA and 76% for PFOS, respectively.[31] Such levels are comparable to background exposure levels that were measured in Danish men in 2008–2009 (mean PFOA: 3.5 ng ml^{-1} and mean PFOS: 8.5 ng ml^{-1}).[32]

In 2003 to 2005, mean DDT and dichlorodiphenyldichloroethylene (*p*, *p*'-DDE) serum levels of South African men living in DDT-sprayed areas were 90.2 and 215.5 μg g^{-1} lipid, respectively;[33] whereas, serum levels of *p*, *p*'-DDE in general populations exposed to *p*, *p*'-DDE as a result of the agricultural use of DDT were 1270 ng g^{-1} lipid (Ukraine) and 580 ng g^{-1} lipid (Poland) in the period 2002–2004[34] and 275 ng g^{-1} lipid in male partners of infertile couples in the period 2000–2001 (USA).[29] The mean serum levels of men from southern and northern Norway in 2001 were somewhat lower: 81 and 66 ng g^{-1} lipid, respectively.[27]

Although serum levels of many of the well-described POPs are now declining, the concentrations of some of the newer compounds, such as perfluorohexanesulfonic acid (PFHxS), perfluorobutanesulfonic acid (PFBS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnA) or their precursors, are increasing.[35,36] Thus, POPs found in the environment today may still be at levels that are potentially harmful to male reproductive health.

Mechanisms

Experimental data suggest that POPs interact with steroid homeostasis and affect hormonal balance through mechanisms involving steroid receptor binding and/or the disruption of steroid biosynthesis or metabolism.[37] Hence, many POPs are regarded as potential endocrine disrupting compounds. Depending on the congener type, PCBs have been demonstrated to exhibit dioxin-like effects, with effects exerted through the aryl hydrocarbon receptor (AhR),[38] in addition to estrogenic, antiestrogenic and antiandrogenic effects.[39,40] The major DDT degradation product *p*, *p*'-DDE is regarded as an androgen receptor antagonist,[41] whereas animal studies suggest both estrogenic and antiandrogenic effects of BFRs.[42,43]

In the following section, current knowledge on the effects of exposure to POPs during adult life and during intrauterine and childhood development on male reproductive health outcomes will be reviewed.

Adult exposure and reproductive health

Due to the potential endocrine-disrupting effects of POPs, an extensive number of epidemiological studies have attempted to clarify whether POPs pose a risk to human male reproductive health.

Polychlorinated biphenyls

The potential harmful effects of PCBs on male reproductive function have been studied extensively. A review of representative studies assessing the potential effects of PCBs on male reproductive health is presented in **Table 1**.

Overall, studies of exposure to PCBs during adulthood indicate some association between PCB and lower sperm motility and to some extent, decreased sperm DNA chromatin integrity and lower levels of free testosterone.

p, p'-Dichlorodiphenyldichloroethylene

In a South African study of a healthy cohort of 311 men living in an endemic malaria area, the mean *p*, *p*'-DDE serum level of men living in DDT-sprayed houses was 239 μg g^{-1} lipid compared with 99.5 μg g^{-1} lipid for men whose houses were not sprayed. The study suggested an inverse association between *p*, *p*'-DDE exposure and semen volume, total sperm count and computer-assisted sperm analysis mean motility, which was partly corroborated by another study of 116 men environmentally exposed to high DDT/*p*, *p*'-DDE levels in Chiapas, Mexico (2000–2001). Here, crude results suggested that increasing plasma *p*, *p*'-DDE concentrations were inversely associated with sperm motility and were positively associated with sperm tail defects and inadequate sperm chromatin condensation.[33,54] Similar to the results on PCBs, background levels of *p*, *p*'-DDE have also been found to be associated with reduced sperm motility.[34] This finding was corroborated by crude but not adjusted analyses by Rignell-Hydbom *et al*.[48] However, other studies did not find any statistically significant associations between *p*, *p*'-DDE and sperm motility.[27,29]

Contradictory to the hypothesized adverse effect of *p*, *p*'-DDE exposure on sperm quality, a few studies have identified positive associations between *p*, *p*'-DDE exposure and sperm concentration. A weak association between *p*, *p*'-DDE and sperm concentration was found in the northern population of a Norwegian study, which also reported a positive association between PCB exposure and sperm concentration.[27] In the study by Toft *et al*.,[34] a positive association between *p*, *p*'-DDE exposure and sperm concentration was observed in a Swedish cohort. This association, however, was suggested to be a chance finding caused by lower than normal sperm concentration in the group with low exposure.[34] Several studies have suggested that *p*, *p*'-DDE is not related to sperm morphology,[29,34,44] sperm DNA integrity,[44,45,47,49] or reproductive hormone levels,[27,52,53,55] although the study by Giwercman *et al*., suggested some effects of *p*, *p*'-DDE exposure on adult male reproductive hormone levels.[22]

Perfluorinated compounds

Results from epidemiological cross-sectional studies of the potential associations between PFCs in adulthood and male reproductive health are conflicting. Some studies suggest effects on sperm morphology, motility and reproductive hormone levels, whereas others report no observed effects. Selected studies are reviewed in **Table 2**. Two cross-sectional studies of men occupationally exposed to PFOA in 1993 and 1995 in the USA found no associations between high serum levels of PFOA and altered reproductive hormone levels.[60] In contrast, studies on background exposure levels of PFOA and PFOS suggest effects on reproductive hormones levels.[32,56,57]

Thus far, only one study has investigated possible associations between PFC exposure and sperm DNA integrity. This study of a cohort of fertile men found that serum PFOA but not PFOS, PFHxS or PFNA in men from Greenland was associated with an increased percentage of (terminal deoxynucleitidyl transferase dUTP nick-end labeling (TUNEL))-positive spermatozoa (increased level of DNA strand breaks), but not an elevated DNA fragmentation index, as assessed by the sperm chromatin structure assay. Additionally, the study suggested that higher levels of spermatozoa positive for the proapoptotic marker Fas were associated with a higher exposure to PFOS in Polish men, whereas only tendencies towards elevated % Fas positivity were indicated for the other regions investigated. Overall, the study did not suggest strong associations between PFOS, PFOA, PFHxS or PFNA and sperm DNA damage or apoptosis.[57] Based on the few studies that have evaluated the possible reproductive health effects of adult exposure to PFCs, it appears that the direction of associations between PFCs and reproductive hormones are not consistent, but that sperm morphology may be targeted by PFC exposure **(Table 2)**.

Brominated flame retardants, dioxin and hexachlorobenzene

Knowledge is sparse as to how adult exposure to BFRs, dioxins and HCBs affects male reproductive health. However, a Japanese study of 10 young men indicated inverse associations between BDE-153 and sperm concentration and testis size,[61] and a Canadian study of 52 men recruited at an infertility clinic indicated adverse effects of BDE-47, BDE-100 and the sum of measured PBDE congeners (BDE-47, BDE-99, BDE-100 and BDE-153) on sperm motility,[62] but no relation with other semen parameters. Finally, a study on 62 men from the USA indicated positive associations between house dust levels of penta-BDEs and serum levels of free and total testosterone, estradiol and sex hormone binding globulin (SHBG) and inverse associations with follicle stimulating hormone (FSH). In the same study, house dust octa-BDEs were positively associated with luteinizing hormone (LH) and testosterone, and finally, deca-BDEs were inversely associated with testosterone.[63] This study expanded a previous report from the same group indicating inverse associations between measured PBDE congeners (47, 99 and 100) in dust samples and free testosterone, LH and FSH and positive associations with SHBG and inhibin B.[64]

Exposure to dioxins during adulthood does not appear to cause long-term effects on semen quality or reproductive hormone levels when measured 20 years later, as suggested in a study of Italian men exposed to high 2,3,7,8-tetrachlorodibenzo-p-dioxin levels when they were 18–26 years of age as a result of the Seveso explosion in 1979.[65] In addition, HCB measurements in adulthood have not been related to changes in sperm quality, sperm DNA integrity or reproductive hormone levels.[49,50,53]

Concluding remarks on adult exposure and persistent organic pollutants

Based on the effects described in these studies, it appears that exposure to PCBs during adult life primarily affects sperm motility, whereas the perfluorinated compounds included here appear to primarily target sperm morphology.

Fetal exposure and reproductive malformations at birth

The possible effects on cryptorchidism and hypospadias of *in utero* exposure to DDT and *p*, *p*'-DDE have been studied in two nested case-referent studies in the USA: the Child Health and Development Study, including 75 cases of cryptorchidism, 66 cases of hypospadias and 283 controls;[66] and the Collaborative Perinatal Project (CPP), including 219 cases of cryptorchidism, 199 cases of hypospadias and 552 controls.[67] Both studies included pregnancies in the period from 1959 to 1966, and both found slightly but not significantly increased risk

Table 1: Epidemiological cross-sectional studies investigating the effects of PCB exposure during adulthood on semen quality and reproductive hormones

Reference	*Study population*	*Exposure*	*Result*	*Outcome*	*Adjustment*	*Statistical analysis*
Semen quality measures						
Haugen *et al.* 2011[27]	197 in total cohort Southern Norway (*n*=95) and Northern Norway (*n*=77)	CB-153	No correlation between CB-153 and progressive motility. Positive correlation between CB-153 and sperm concentration in the northern cohort	Sperm motility → Sperm concentration ↑ Sperm morphology -	Unadjusted	Pearson's correlation coefficient
Bonde *et al.* 2008[44]	798 in total 198 Greenlandic, 191 Swedish, 208 Ukrainian and 198 Polish men	CB-153	Progressive motility (%) was inversely related to serum CB-153 among Inuits and European men	Sperm motility ↓ Sperm concentration → Sperm morphology →	In (period of abstinence)	Multiple linear regression
Stronati *et al.* 2006[45]	652 in total 200 Greenlandic, 166 Swedish, 152 Ukrainian and 134 Polish men	CB-153	Significant positive association between CB-153 and % TUNEL positivity and % Bcl-xL-positive sperm cells in the combined European cohorts but not in Inuits	Sperm motility - Sperm concentration - Sperm morphology - Sperm DNA integrity ↓	In (period of abstinence) and In (age)	Multiple linear regression
Toft *et al.* 2006[34]	763 in total 194 Greenlandic, 185 Swedish, 195 Ukrainian and 189 Polish men	CB-153	Sperm motility was inversely related to CB-153 concentration in Greenland and Sweden populations and in the total cohort. Positive association between CB-153 and sperm concentration in the Swedish but not the other cohorts	Sperm motility ↓ Sperm concentration ↑ Sperm morphology →	Unadjusted because none of the potential confounders changed the regression coefficients or means by more than 10%. In the analysis across all four regions, population was included as a covariate	Linear and multiple linear regression
Rignell-Hydbom *et al.* 2005[46]	176 Swedish fishermen	CB-153	Crude significant positive association between CB-153 and % DFI (disappears when adjusting for age). Quintile with the lowest CB-153 exposure had significantly lower % DFI than the other quintiles	Sperm motility - Sperm concentration - Sperm morphology - Sperm DNA integrity ↓		Multiple linear regression
Spano *et al.* 2005[47]	707 in total 193 Greenlandic, 178 Swedish, 195 Ukrainian and 141 Polish men	CB-153	Significant positive association between CB-153 and % DFI in the Swedish, Ukrainian and combined European cohorts	Sperm motility - Sperm concentration - Sperm morphology - Sperm DNA integrity ↓	Study group (European men only), period of abstinence and age	Multiple linear regression
Rignell-Hydbom *et al.* 2004[48]	195 Swedish fishermen (99 West coast and 96 East coast)	CB-153	Significant inverse association between CB-153 and progressively motile spermatozoa in unadjusted analyses. After adjusting for age, the association was no longer significant	Sperm motility → Sperm concentration → Sperm morphology -	Age	Pearson's correlation coefficient and multiple linear regression
Hauser *et al.* 2003[29]	212 US male partners of infertile couples	PCB-118,138,153, ∑estrogenic PCBs, ∑dioxin like PCBs, ∑enzyme inducing PCBs and ∑PCBs	Significant inverse association between PCB-138 and below-reference sperm motility and sperm morphology. Significant inverse relationship between continuous ln sperm concentration and PCB-138	Sperm motility ↓ Sperm concentration ↓ Sperm morphology ↓	Age, smoking and period of abstinence	Multivariate logistic regression analysis and multiple linear regression
Hauser *et al.* 2003[49]	212 US male partners of infertile couples	PCB-118,138,153, ∑estrogenic PCBs, ∑dioxin like PCBs, ∑enzyme inducing PCBs and ∑PCBs	No significant associations between PCB and comet assay parameters	Sperm motility - Sperm concentration - Sperm morphology - Sperm DNA integrity →	Age, smoking	Multiple regression
Richthoff *et al.* 2003[28]	305 Swedish men	CB-153	Weak but statistically significant negative correlation between CB-153 levels and CASA sperm motility	Sperm motility ↓ Sperm concentration → Sperm morphology -	Unadjusted because adjusted estimates differed less than 15% from the crude estimate	Pearson's correlation coefficient and linear regression

(to be continued)...

Table 1: Continued

Reference	*Study population*	*Exposure*	*Result*	*Outcome*	*Adjustment*	*Statistical analysis*
Dallinga *et al.* 2002[50]	65 Dutch male partners of infertile couples	PCB-118, 138, 153, 180, ∑PCB, PCB metabolites and ∑PCB metabolites	Significant negative correlation between ∑PCB metabolites and progressively motile sperm concentration (PMSC) and total sperm count for the female factor subfertility group. Significant positive association between individual and combined PCB and sperm morphology	Sperm motility ↓ Total sperm count ↓ Sperm morphology ↑	Unadjusted	Linear regression
Rozati *et al.* 2002[51]	53 Indian male partners of infertile couples	PCB	Significant inverse association between seminal PCB concentration and total progressive motility. Significant positive correlation between seminal PCB and % single-stranded sperm DNA	Sperm motility ↓ Sperm concentration → Sperm morphology → Sperm DNA integrity ↓	Unadjusted	Linear regression
Reproductive hormones						
Haugen *et al.* 2011[27]	197 in total Southern Norway (*n*=95) and northern Norway (*n*=77)	CB-153	Significant positive association between CB-153 and SHBG in the total cohort and both regional cohorts	Free testosterone → Testosterone (total) → SHBG ↑ Estradiol → Inhibin B → FSH → LH →	Age and BMI	Multiple regression
Goncharov *et al.* 2009[52]	257 Mohawk US men (with moderately elevated PCB levels)	PCB-52, 74, 99, 105, 118, 138, 153, 170, 180, 201, 203, 206, mono-, di-, tri- and tetra-*ortho*, dioxin-like and ∑PCBs	Significant inverse association between testosterone and highest versus lowest ∑PCB tertile. Significant associations between testosterone and PCB-74, 99, 153, 206 and congener groups (mono-*ortho*, di-*ortho*, tri-and tetra-*ortho*-substituted and dioxin-like PCBs). No other reproductive hormones were measured	Testosterone ↓	Age, BMI, total serum lipids, concentrations of HCB, DDE and Mirex	Logistic regression
Bonde *et al.* 2008[44]	874 in total 325 Greenlandic, 190 Swedish, 215 Ukrainian and 144 Polish men	CB-153	Significant positive association between CB-153 and SHBG and a significant inverse association between CB-153 and free testosterone among European men. Significant positive association between CB-153 and LH in Inuit men	Free testosterone ↓ Testosterone (total) - SHBG ↑ Estradiol- Inhibin B → FSH → LH ↑	Time of blood sampling, ln age (years)	Multiple regression
Giwercman *et al.* 2006[22]	749 in total 258 Greenlandic, 184 Swedish, 194 Ukrainian and 113 Polish men	CB-153	Significant positive association between CB-153 and SHBG and LH in the Ukrainian cohort. In Greenland, the highest exposure group exhibited significantly higher LH levels compared with the reference group. No significant associations between CB-153 and any of the reproductive hormones in the Swedish or pooled data cohorts. Free testosterone levels were significantly lower in the third highest CB-153 group compared with the reference in Poland	SHBG ↑ LH ↑ Free testosterone ↓ Estradiol → Inhibin B → FSH →	BMI, season, time of blood sampling, age, alcohol and smoking	Multiple linear regression
Richthoff *et al.* 2003[28]	305 Swedish men	CB-153	Significant positive correlation between CB-153 and SHBG Significant inverse relationship between CB-153 and the testosterone: SHBG ratio	Free testosterone ↓ Testosterone (total) → SHBG ↑ Estradiol → Inhibin B → FSH → LH →	BMI	Pearson's correlation coefficient and multiple linear regression
Hagmar *et al.* 2001[53]	43 Swedish men and 67 Latvian men	PCB 105, 118, 129, 138, 146, 153, 156, 167, 170, 172, 177, 180, 183, 187, 194, 195, 196, 5 OH-PCBs, ∑PCBs and ∑OH-PCBs	Weak inverse associations between free testosterone and ∑PCB and ∑OH-PCB (crude results) (did not remain statistically significant after adjusting for age)	Free testosterone → Testosterone (total) - SHBG - Estradiol - Inhibin B - FSH → LH →	Age	Multiple linear regression

BMI: body mass index; CB-153: 2,2',4,4',5,5'-hexachlorobiphenyl; DDE: dichlorodiphenyldichloroethylene; DFI: DNA fragmentation index; FSH: follicle-stimulating hormone; HCB: hexachlorobenzene; LH: luteinizing hormone; PCB: polychlorinated biphenyl; SHBG: sex hormone-binding globulin; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling

Table 2: A review of selected epidemiological cross-sectional studies investigating the effects of PFC exposure during adulthood on semen quality and reproductive hormones

Reference	*Study population*	*Exposure*	*Result*	*Outcome*	*Adjustment*	*Statistical analysis*
Joensen *et al.* 2013[32]	247 Danish men considered for military service	PFHxS, PFHpS, PFOS, PFOA, PFNA and PFDA	PFOS was inversely associated with testosterone, free testosterone, FAI, testosterone/LH, free testosterone/LH and FAI/LH. PFHpS was inversely associated with the percentage of progressively motile spermatozoa	Sperm motility ↓ Sperm concentration → Sperm morphology → Sperm DNA integrity - Free testosterone ↓ Testosterone (total) ↓ SHBG → Estradiol → Inhibin B → FSH → LH →	Sperm concentration and total sperm count were adjusted for abstinence time; progressive motility was adjusted for time to semen analysis; morphology was not adjusted; and testosterone, free testosterone, SHBG, estradiol, FSH and LH were adjusted for BMI and smoking	Multiple linear regression
Raymer *et al.* 2012[56]	256 male partners of infertile couples	PFOA and PFOS	No association between PFOA or PFOS and any of the semen quality measures. PFOA was positively correlated with free testosterone and LH	Sperm motility → Sperm concentration → Sperm morphology - Sperm DNA integrity - Free testosterone ↑ Testosterone (total) → SHBG - Estradiol → Inhibin B - FSH → LH ↑	Sperm motility and sperm concentration were adjusted for age, period of abstinence and tobacco use. Associations between PFOA and PFOS and the reproductive hormones were unadjusted	Multiple linear regression Spearman correlation coefficient
Specht *et al.* 2012[57]	548 in total. 198 Greenlandic men, 207 Ukrainian men and 143 Polish men	PFOS, PFOA, PFNA, PFHxS, PFDA, PFUnDA, and PFDoDA	No significant associations between PFOS, PFOA, PFNA or PFHxS and %DFI (all regions). PFOA was positively associated with the percentage of TUNEL-positive sperm cells for men from Greenland (only in trend analysis). Higher PFOS was positively associated with the proapoptotic marker Fas (significant for Polish men). Significant positive association between PFOA and SHBG in Polish and Greenlandic men (after adjustment, this was no longer significant in Greenlandic men)	Sperm motility - Sperm concentration - Sperm morphology - Sperm DNA integrity ↓ (TUNEL assay, only Greenland) Free testosterone - Testosterone (total) → SHBG ↑ Estradiol → Inhibin B → FSH → LH →	%DFI, Fas, Bcl-xL and reproductive hormones were adjusted for age, BMI, caffeinated drinks, cotinine, fever, spillage, abstinence time, genital infections and testicular disorders	Multiple general linear models
Toft *et al.* 2012[58]	588 in total. 196 Greenlandic men, 203 Ukrainian men and 189 Polish men	PFOS, PFOA, PFHxS and PFNA	Significantly higher sperm concentration and total sperm concentration in the second PFOS tertile compared with the first PFOS tertile for Polish men. Significant inverse association between PFOS and the proportion of morphologically normal spermatozoa in the total cohort. Significantly increased proportion of tail defects in the second PFOS tertile compared to the first PFOS tertile. Significant positive association between PFOA and the proportion of motile sperm in Greenlandic men and in the total cohort. PFHxS significantly inversely associated with the percentage of morphologically normal spermatozoa (total cohort)	Sperm motility ↑ Sperm concentration ↑ Sperm morphology ↓ Sperm DNA integrity - Free testosterone - Testosterone (total) - SHBG - Estradiol - Inhibin B - FSH - LH -	Adjustment for age, abstinence time, spillage, smoking, urogenital infections and BMI	Multiple linear regression
Joensen, *et al.* 2009[59]	105 Danish men reporting for military draft. Sampled on the basis of high (53 men) or low testosterone levels (52 men)	PFOA and PFOS	Significant inverse associations between PFOA and PFOS combined and the percentage of morphologically normal spermatozoa and the total number of normal spermatozoa	Sperm motility → Sperm concentration → Sperm morphology ↓ Sperm DNA integrity - Free testosterone → Testosterone (total) → SHBG → Estradiol → Inhibin B → FSH → LH →	Reproductive hormones were adjusted for time of blood sampling; volume, sperm concentration, total sperm count and total morphologically normal sperm count were adjusted for duration of abstinence; motility was adjusted for time to semen analysis; and morphology was not adjusted for confounders	Multiple linear regression

DFI: DNA fragmentation index; FAI: free androgen index; LH: luteinizing hormone; PFHpS: perfluoroheptane sulfonic acid; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctane sulfonate; PFDA: perfluorodecanoic acid; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFUnDA: perfluoroundecanoic acid; PFDoDA: perfluorododecanoic acid; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling

of cryptorchidism and hypospadias, with odds ratios of approximately 1.3 at the highest maternal *p*, *p*'-DDE exposure level compared to the lowest maternal *p*, *p*'-DDE exposure level. Furthermore, a recent Swedish study on 237 hypospadias cases compared with a similar number of controls suggested that increased *p*, *p*'-DDE and HCB exposure, but not PCB exposure may be associated with an increased risk of hypospadias.[68]

No association between placenta concentrations of PCB and dioxins and the risk of cryptorchidism was observed in a Danish-Finnish study.[69] Although no evidence of an association between PCB or HCB exposure and cryptorchidism was suggested, an increased risk of hypospadias was suggested at high PCB exposure levels in the CPP in the USA.[70,71]

A few studies have used breast milk as a proxy for *in utero* exposure, and a French study found an association between breast milk PCB concentrations and cryptorchidism.[72] Additionally, a Danish-Finnish study found an increased risk of cryptorchidism at higher breast milk levels of PBDE, but did not find any associations with placental PBDE levels.[73]

Based on such small and frequently nonstatistically significant effects, it remains inconclusive whether persistent organic compounds at environmental exposure levels increase the risk of hypospadias and cryptorchidism, but it seems that, at most, a small increased risk can be ascribed to exposure to these compounds.

Another outcome indicating feminization of males is decreased anogenital distance, as females have a shorter anogenital distance than males. This outcome was more sensitive to exposure to environmental antiandrogens than hypospadias and cryptorchidism in animal studies.[74] Thus, although the clinical consequences of reduced anogenital distance are probably limited, it may be a sensitive marker of endocrine disturbances in humans. In the USA a smaller study including 37 male offspring indicated reduced anogenital distance at higher *p*, *p*'-DDE exposure.[75] However, a larger study among 781 mother-child pairs in Chiapas, Mexico, of which 29% reported living in DDT-sprayed homes, indicated no association between *p*, *p*'-DDE exposure and anogenital distance or penile length, suggesting that even high exposure to *p*, *p*'-DDE does not seem to have a significant impact on these outcomes in humans.[76]

Perinatal exposure and reproductive health in adulthood

A large number of studies have investigated possible male reproductive health effects of exposure to POPs during adult life. However, only a few studies have had the opportunity to investigate the very core of the endocrine disruptor hypothesis, namely, the possible long-term effects of exposure to compounds with endocrine-disrupting effects during fetal life on male reproductive health.

Exposure to endocrine-modulating agents during fetal life is of particular concern because an imbalance of the fetal hormonal environment may affect the normal development of male reproductive organs, which may cause long-term effects on the male reproductive system.

The Yu-Cheng oil-disease in Taiwan region in 1979, where over 2000 men and women were accidently contaminated with high levels of PCBs and their pyrolytic products (e.g. polychlorinated dibenzofurans), has provided valuable knowledge regarding how high exposures to POPs during fetal development affect adult human male reproductive health. Comparisons between prenatally exposed men ($n = 12$) and unexposed controls ($n = 23$) demonstrated increased abnormal morphology and a reduced percentage of progressively motile and rapidly motile spermatozoa in prenatally exposed men compared with controls. Additionally, spermatozoa from exposed men exhibited reduced oocyte penetration capacity compared with controls.[77]

Another study that assessed the effects of accidental perinatal exposure to dioxin as a result of the trichlorophenol plant explosion near Seveso, Italy (1976) indicated permanent effects on sperm quality.[78] The extrapolated median serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin concentration of the mothers at the time of conception was 26 parts per trillion. In this study, the background levels of dioxin exposure were assumed to be 10 parts per trillion, and serum concentrations in breastfed sons were considered to be twofold that of the mothers. The authors found that the 39 men exposed *in utero* and postnatally through breastfeeding exhibited significantly lower sperm concentrations, total sperm counts, numbers of total motile spermatozoa and inhibin B concentrations and higher FSH concentrations compared with the controls. There were no differences in any of the semen parameters or reproductive hormones between the formula-fed men only exposed to dioxin *in utero* ($n = 18$) and the controls. Hence, the male reproductive system seems to be sensitive to perinatal dioxin exposure rather than *in utero* exposure alone.[78]

A recent follow-up study of a cohort of 169 Danish men who participated in a study in 2008–2009 when they were 19 to 21 years-old addressed the potential long-term effects of *in utero* exposure to background levels of the perfluorinated compounds PFOA and PFOS on adult male reproductive health. The study indicated inverse associations between PFOA and sperm concentrations and total sperm counts and that higher exposure to PFOA during fetal development was associated with higher levels of LH and FSH in adulthood, whereas no associations between PFOS and any of the semen parameters or reproductive hormones were found.[79]

Based on the limited number of studies that have investigated the potential harmful effects of *in utero* exposure to POPs, some indications of long-term effects of exposure during fetal development of the reproductive organs are evident and warrant further studies to support the findings.

QUESTIONS FROM THE PANEL

Q1: Can the endocrine disruption hypothesis explain the observed effects of environmental exposures on male reproductive health?

A1: The endocrine disruption hypothesis states that various environmental chemicals at low levels and possibly in combination with each other might interfere with hormone signaling, especially when exposure occurs during critical phases of fetal and childhood development.[80] Although the hypothesis has been corroborated in experimental animal studies, evidence from the human population remains limited. As described previously in this paper, exposures to environmental contaminants seem to have, at most, weak effects on malformations of the reproductive organs, puberty development and adult male semen quality. However, recent studies add evidence that exposure during fetal and early postnatal life in particular may have long-term consequences for male semen quality.[78,79] The scarcity of studies relating fetal exposure to adult semen quality is most likely primarily related to the fact that only a few cohorts exist with stored blood samples from mothers during pregnancy and with offspring of sufficient age to perform follow-up studies. Therefore, although most studies to date have provided only limited evidence that environmental chemicals acting as endocrine disruptors can contribute to the observed poor male fertility, one of the core elements of the endocrine disruptor hypothesis has remained untested for almost 20 years, and additional evidence for or against this hypothesis will only slowly be accumulated in the coming years. However, it should be noted that male fertility

may be affected by several other factors in addition to environmental contaminants, including prenatal vitamin deficiencies, infections and medications, which have well-known effects on human development and may be harmful to male fertility as well. Therefore, endocrine disruption is one factor among a list of factors that may affect human fertility, and thus, other factors should be given equal priority in epidemiological research.

Q2: Is pubertal development part of 'reproductive development'?

A2: Pubertal development is regarded as an important part of the reproductive development and delayed or accelerated pubertal development have been suggested to be associated with environmental exposures during the fetal and/or childhood period.

For boys, some studies report a decline in the age at reaching Tanner stage 2 for genital development and pubic hair development, but because pubertal timing is less noticeable than menarche onset in females, more uncertainty exists with respect to the time trend of puberty in boys.[81,82]

Diverging associations between exposure to organochlorines and pubertal timing have been reported, including delayed puberty in PCB-, dioxin- and PFOS-exposed boys;[83–86] whereas, *in utero* PCB but not *p*, *p*'-DDE exposure may be related to earlier puberty development.[87,88]

Thus, the possible effects of fetal and childhood exposure on male reproductive development are not entirely consistent.

Q3: How can epidemiological studies take multiple exposures at the same time into account?

A3: Recent animal studies clearly demonstrate that combined exposures can produce effects even when each individual exposure is below the lowest observed exposure level,[89] and therefore, it is important to give additional attention to how to evaluate multiple exposures in human epidemiological studies.

With advances in the methods for chemical assessments, it has become simpler and less expensive to measure a wealth of chemicals in small volumes of biomaterials and large sample sizes. Therefore, increasing amounts of data have recently become available, allowing us to start modeling the effects of combined exposures in human studies.

Traditionally, epidemiological studies focus on the effects of single compounds on the studied health outcomes, and if information on additional exposures is available, the studied association is often adjusted for these exposures.

However, if the concentration of the single compound is too low to induce an effect on the studied outcome, this association may be overlooked by the single chemical approach. Furthermore, the single chemical approach has the weakness that multiple testing inflates the risk of chance findings, and associations between single chemicals and outcomes cannot often be replicated in future studies.

Combining exposures in a sensible way is not an easy task. Often, we only know the mechanisms of action for a few of the measured compounds, and adding exposures with different effects may blur associations instead of giving a better estimate of combined effects. To date, authors have mainly added compounds of similar chemical classes (e.g., sum of PCBs) or used more mechanistic approaches (e.g., the sum of estrogenic PCBs vs the sum of antiestrogenic PCBs).

Models adding different groups of chemicals that all act on one outcome are needed in future studies to evaluate whether the sum of multiple exposures at low levels is harmful to human reproductive health, which cannot be ruled out based on studies of individual chemicals.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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