



Open Access

## INVITED REVIEW

Reproductive Health

# Persistent organic pollutants and male reproductive health

Anne Vested<sup>1</sup>, Aleksander Giwercman<sup>2</sup>, Jens Peter Bonde<sup>3</sup>, Gunnar Tøft<sup>1</sup>

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.

*Asian Journal of Andrology* (2014) 16, (71–80); doi: 10.4103/1008-682X.122345; published online: 16 December 2013

**Keywords:** endocrine disruption; male reproduction; persistent organic compounds; reproductive hormones; semen quality

## INTRODUCTION

The possible effects of exposure to environmental contaminants on male reproductive function have been addressed in a number of epidemiological studies.<sup>1–3</sup> 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,<sup>4–6</sup> 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.<sup>7</sup>

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<sup>8</sup> 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.<sup>9</sup> 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.<sup>10–12</sup>

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

<sup>1</sup>Department of Occupational Medicine, Danish Ramazzini Centre, Aarhus University Hospital, Aarhus, Denmark; <sup>2</sup>Reproductive Medicine Centre, Lund University, Skane University Hospital, Malmö, Sweden; <sup>3</sup>Department of Occupational and Environmental Medicine, Bispebjerg Hospital of Copenhagen University, Copenhagen, Denmark.

Correspondence: A Vested ([anneved@rm.dk](mailto:anneved@rm.dk))

Received: 01-05-2013; Revised: 13-08-2013; Accepted: 16-09-2013

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<sup>-1</sup>.<sup>13-15</sup> 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.<sup>13</sup> 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.<sup>16</sup>

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.<sup>17,18</sup> In addition, male anogenital distance has recently been demonstrated to be related to semen quality and chance of fatherhood<sup>19,20</sup> 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.<sup>21</sup> Finally, reproductive hormone levels are considered markers for male reproductive function.<sup>22</sup>

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.<sup>2</sup>

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.<sup>23</sup>

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  $\Sigma$ PCB and CB-153 are highly correlated.<sup>24,25</sup> 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.<sup>26</sup>

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<sup>-1</sup> lipid,<sup>27,28</sup> which is similar to mean levels in the USA during the same time period (44 ng g<sup>-1</sup> lipid).<sup>29</sup> 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.<sup>23,30</sup> 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<sup>-1</sup>, respectively, and between 2001 and 2010, these levels declined 48% for PFOA and 76% for PFOS, respectively.<sup>31</sup> Such levels are comparable to background exposure levels that were measured in Danish men in 2008–2009 (mean PFOA: 3.5 ng ml<sup>-1</sup> and mean PFOS: 8.5 ng ml<sup>-1</sup>).<sup>32</sup>

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  $\mu$ g g<sup>-1</sup> lipid, respectively;<sup>33</sup> 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<sup>-1</sup> lipid (Ukraine) and 580 ng g<sup>-1</sup> lipid (Poland) in the period 2002–2004<sup>34</sup> and 275 ng g<sup>-1</sup> lipid in male partners of infertile couples in the period 2000–2001 (USA).<sup>29</sup> The mean serum levels of men from southern and northern Norway in 2001 were somewhat lower: 81 and 66 ng g<sup>-1</sup> lipid, respectively.<sup>27</sup>

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.<sup>35,36</sup> 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.<sup>37</sup> 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),<sup>38</sup> in addition to estrogenic, antiestrogenic and antiandrogenic effects.<sup>39,40</sup> The major DDT degradation product *p,p'*-DDE is regarded as an androgen receptor antagonist,<sup>41</sup> whereas animal studies suggest both estrogenic and antiandrogenic effects of BFRs.<sup>42,43</sup>

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  $\mu\text{g g}^{-1}$  lipid compared with 99.5  $\mu\text{g g}^{-1}$  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.<sup>33,54</sup> Similar to the results on PCBs, background levels of *p*, *p'*-DDE have also been found to be associated with reduced sperm motility.<sup>34</sup> This finding was corroborated by crude but not adjusted analyses by Rignell-Hydbom *et al.*<sup>48</sup> However, other studies did not find any statistically significant associations between *p*, *p'*-DDE and sperm motility.<sup>27,29</sup>

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.<sup>27</sup> In the study by Toft *et al.*,<sup>34</sup> 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.<sup>34</sup> Several studies have suggested that *p*, *p'*-DDE is not related to sperm morphology,<sup>29,34,44</sup> sperm DNA integrity,<sup>44,45,47,49</sup> or reproductive hormone levels,<sup>27,52,53,55</sup> although the study by Giwercman *et al.*, suggested some effects of *p*, *p'*-DDE exposure on adult male reproductive hormone levels.<sup>22</sup>

### 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.<sup>60</sup> In contrast, studies on background exposure levels of PFOA and PFOS suggest effects on reproductive hormones levels.<sup>32,56,57</sup>

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 deoxynucleotidyl 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.<sup>57</sup> 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,<sup>61</sup> 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,<sup>62</sup> 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.<sup>63</sup> 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.<sup>64</sup>

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.<sup>65</sup> In addition, HCB measurements in adulthood have not been related to changes in sperm quality, sperm DNA integrity or reproductive hormone levels.<sup>49,50,53</sup>

### 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;<sup>66</sup> and the Collaborative Perinatal Project (CPP), including 219 cases of cryptorchidism, 199 cases of hypospadias and 552 controls.<sup>67</sup> 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
<b>Semen quality measures</b>						
Haugen <i>et al.</i> 2011 <sup>27</sup>	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 <i>et al.</i> 2008 <sup>44</sup>	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 <i>et al.</i> 2006 <sup>45</sup>	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 <i>et al.</i> 2006 <sup>34</sup>	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 <i>et al.</i> 2005 <sup>46</sup>	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 <i>et al.</i> 2005 <sup>47</sup>	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 <i>et al.</i> 2004 <sup>48</sup>	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 <i>et al.</i> 2003 <sup>29</sup>	212 US male partners of infertile couples	PCB-118,138,153, Σestrogenic PCBs, Σdioxin like PCBs, Σenzyme inducing PCBs and ΣPCBs	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 <i>et al.</i> 2003 <sup>49</sup>	212 US male partners of infertile couples	PCB-118,138,153, Σestrogenic PCBs, Σdioxin like PCBs, Σenzyme inducing PCBs and ΣPCBs	No significant associations between PCB and comet assay parameters	Sperm motility - Sperm concentration - Sperm morphology - Sperm DNA integrity →	Age, smoking	Multiple regression
Richthoff <i>et al.</i> 2003 <sup>28</sup>	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 <i>et al.</i> 2002 <sup>50</sup>	65 Dutch male partners of infertile couples	PCB-118, 138, 153, 180, ΣPCB, PCB metabolites and ΣPCB metabolites	Significant negative correlation between ΣPCB 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 <i>et al.</i> 2002 <sup>51</sup>	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
<b>Reproductive hormones</b>						
Haugen <i>et al.</i> 2011 <sup>27</sup>	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 <i>et al.</i> 2009 <sup>52</sup>	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 ΣPCBs	Significant inverse association between testosterone and highest versus lowest ΣPCB 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 <i>et al.</i> 2008 <sup>44</sup>	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, In age (years)	Multiple regression
Giwercman <i>et al.</i> 2006 <sup>22</sup>	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 <i>et al.</i> 2003 <sup>28</sup>	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 <i>et al.</i> 2001 <sup>53</sup>	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, ΣPCBs and ΣOH-PCBs	Weak inverse associations between free testosterone and ΣPCB and ΣOH-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 <i>et al.</i> 2013 <sup>32</sup>	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 <i>et al.</i> 2012 <sup>56</sup>	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 <i>et al.</i> 2012 <sup>57</sup>	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 <i>et al.</i> 2012 <sup>58</sup>	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, <i>et al.</i> 2009 <sup>59</sup>	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.<sup>68</sup>

No association between placenta concentrations of PCB and dioxins and the risk of cryptorchidism was observed in a Danish-Finnish study.<sup>69</sup> 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.<sup>70,71</sup>

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.<sup>72</sup> 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.<sup>73</sup>

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.<sup>74</sup> 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.<sup>75</sup> 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.<sup>76</sup>

#### **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 accidentally 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.<sup>77</sup>

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.<sup>78</sup> 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.<sup>78</sup>

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.<sup>79</sup>

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.<sup>80</sup> 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.<sup>78,79</sup> 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.<sup>81,82</sup>

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

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,<sup>89</sup> 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.

## REFERENCES

- 1 Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, *et al*. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J Toxicol Environ Health B Crit Rev* 2008;11: 373-517.
- 2 Sharpe RM. Environmental/lifestyle effects on spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* 2010;365: 1697-712.
- 3 Toft G, Hagmar L, Giwercman A, Bonde JP. Epidemiological evidence on reproductive effects of persistent organochlorines in humans. *Reprod Toxicol* 2004;19: 5-26.
- 4 Gray LE Jr. Xenoendocrine disrupters: laboratory studies on male reproductive effects. *Toxicol Lett* 1998;102-103: 331-5.
- 5 Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, *et al*. Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. *Biol Reprod* 2002;66: 272-81.
- 6 Toft G, Guillette LJ Jr. Decreased sperm count and sexual behavior in mosquitofish exposed to water from a pesticide-contaminated lake. *Ecotoxicol Environ Saf* 2005; 60: 15-20.
- 7 Varghese AC, du Plessis SS, Agarwal A. Male gamete survival at stake: causes and solutions. *Reprod Biomed Online* 2008;17: 866-80.
- 8 El-Shahawi MS, Hamza A, Bashammakh AS, Al-Saggaf WT. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Talanta* 2010;80: 1587-97.
- 9 Kissa Erik. Fluorinated surfactants and repellents. 2nd edition, Surfactant science series volume 97, New York: Marcel Dekker; 2001.
- 10 Bonde JP, Joffe M, Apostoli P, Dale A, Kiss P, *et al*. Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. *Occup Environ Med* 2002; 59: 234-42.
- 11 Siu ER, Mruk DD, Porto CS, Cheng CY. Cadmium-induced testicular injury. *Toxicol Appl Pharmacol* 2009; 238: 240-9.
- 12 Sheiner EK, Sheiner E, Hammel RD, Potashnik G, Carel R. Effect of occupational exposures on male fertility: literature review. *Ind Health* 2003; 41: 55-62.
- 13 Bonde JP, Ernst E, Jensen TK, Hjøllund NH, Kolstad H, *et al*. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 1998;352: 1172-7.
- 14 Slama R, Eustache F, Ducot B, Jensen TK, Jørgensen N, *et al*. Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Hum Reprod* 2002;17: 503-15.
- 15 Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, *et al*. Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 2001;345: 1388-93.
- 16 Spano M, Bonde JP, Hjøllund HI, Kolstad HA, Cordelli E, *et al*. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. *Fertil Steril* 2000; 73: 43-50.
- 17 Hutson JM, Balic A, Nation T, Southwell B. Cryptorchidism. *Semin Pediatr Surg* 2010;19: 215-24.
- 18 Wang MH, Baskin LS. Endocrine disruptors, genital development, and hypospadias. *J Androl* 2008;29: 499-505.
- 19 Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI. The relationship between anogenital distance, fatherhood, and fertility in adult men. *PLoS One* 2011;6: e18973.
- 20 Mendiola J, Stahlhut RW, Jørgensen N, Liu F, Swan SH. Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. *Environ Health Perspect* 2011;119: 958-63.
- 21 Golub MS, Collman GW, Foster PM, Kimmel CA, Rajpert-De Meyts E, *et al*. Public health implications of altered puberty timing. *Pediatrics* 2008; 121 Suppl 3: S218-30.
- 22 Giwercman AH, Rignell-Hydbom A, Toft G, Rylander L, Hagmar L, *et al*. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: a study of inuit and three European cohorts. *Environ Health Perspect* 2006;114: 1348-53.
- 23 D'eon JC, Mabury SA. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ Sci Technol* 2011;45: 7974-84.
- 24 Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Strömberg U, *et al*. Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation, and fish consumption. *Arch Environ Contam Toxicol* 1997;32: 329-36.
- 25 Glynn AW, Wolk A, Aune M, Atuma S, Zettermark S, *et al*. Serum concentrations of organochlorines in men: a search for markers of exposure. *Sci Total Environ* 2000; 263: 197-208.
- 26 Gasull M, Bosch de Basea M, Puigdomenech E, Pumarega J, Porta M. Empirical analyses of the influence of diet on human concentrations of persistent organic pollutants: a systematic review of all studies conducted in Spain. *Environ Int* 2011;37: 1226-35.
- 27 Haugen TB, Tefre T, Malm G, Jönsson BA, Rylander L, *et al*. Differences in serum levels of CB-153 and *p*, *p'*-DDE, and reproductive parameters between men living south and north in Norway. *Reprod Toxicol* 2011;32: 261-7.
- 28 Richthoff J, Rylander L, Jönsson BA, Akesson H, Hagmar L, *et al*. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive



- function in young males from the general Swedish population. *Environ Health Perspect* 2003;111: 409–13.
- 29 Hauser R, Chen Z, Pothier L, Ryan L, Altshul L. The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p, p'-DDE. *Environ Health Perspect* 2003;111: 1505–11.
- 30 Haug LS, Thomsen C, Becher G. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ Sci Technol* 2009;43: 2131–6.
- 31 Olsen GW, Lange CC, Ellefson ME, Mair DC, Church TR, *et al*. Temporal trends of perfluoroalkyl concentrations in american red cross adult blood donors, 2000–2010. *Environ Sci Technol* 2012;46: 6330–8.
- 32 Joensen UN, Veyrand B, Antignac JP, Blomberg Jensen M, Petersen JH, *et al*. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. *Hum Reprod* 2013;28: 599–608.
- 33 Aneek-Hahn NH, Schulerburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. *J Androl* 2007;28: 423–34.
- 34 Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A, *et al*. Semen quality and exposure to persistent organochlorine pollutants. *Epidemiology* 2006;17: 450–8.
- 35 Glynn A, Berger U, Bignert A, Ullah S, Aune M, *et al*. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. *Environ Sci Technol* 2012;46: 9071–9.
- 36 Olsen GW, Ellefson ME, Mair DC, Church TR, Goldberg CL, *et al*. Analysis of a homologous series of perfluorocarboxylates from American Red Cross adult blood donors, 2000–2001 and 2006. *Environ Sci Technol* 2011;45: 8022–9.
- 37 Sharpe RM, Irvine DS. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *BMJ* 2004;328: 447–51.
- 38 White SS, Birnbaum LS. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2009;27: 197–211.
- 39 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.
- 40 Ulbrich B, Stahlmann R. Developmental toxicity of polychlorinated biphenyls (PCBs): a systematic review of experimental data. *Arch Toxicol* 2004;78: 252–68.
- 41 Kelce WR, Stone CR, Laws SC, Gray LE, Kempainen JA, *et al*. Persistent DDT metabolite p, p'-DDE is a potent androgen receptor antagonist. *Nature* 1995;375: 581–5.
- 42 Legler J. New insights into the endocrine disrupting effects of brominated flame retardants. *Chemosphere* 2008;73: 216–22.
- 43 Meerts IA, Letcher RJ, Hoving S, Marsh G, Bergman A, *et al*. *In vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. *Environ Health Perspect* 2001;109: 399–407.
- 44 Bonde JP, Toft G, Rylander L, Rignell-Hydbom A, Giwercman A, *et al*. INUENDO. Fertility and markers of male reproductive function in Inuit and European populations spanning large contrasts in blood levels of persistent organochlorines. *Environ Health Perspect* 2008;116: 269–77.
- 45 Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, *et al*. Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of CB-153 and p, p'-DDE in European and Inuit populations. *Reproduction* 2006;132: 949–58.
- 46 Rignell-Hydbom A, Rylander L, Giwercman A, Jönsson BA, Lindh C, *et al*. Exposure to PCBs and p, p'-DDE and human sperm chromatin integrity. *Environ Health Perspect* 2005;113: 175–9.
- 47 Spano M, Toft G, Hagmar L, Eleuteri P, Rescia M, *et al*. INUENDO. Exposure to PCB and p, p'-DDE in European and Inuit populations: impact on human sperm chromatin integrity. *Hum Reprod* 2005;20: 3488–99.
- 48 Rignell-Hydbom A, Rylander L, Giwercman A, Jonsson BA, Nilsson-Ehle P, *et al*. Exposure to CB-153 and p, p'-DDE and male reproductive function. *Hum Reprod* 2004;19: 2066–75.
- 49 Hauser R, Singh NP, Chen Z, Pothier L, Altshul L. Lack of an association between environmental exposure to polychlorinated biphenyls and p, p'-DDE and DNA damage in human sperm measured using the neutral comet assay. *Hum Reprod* 2003;18: 2525–33.
- 50 Dallinga JW, Moonen EJ, Dumoulin JC, Evers JL, Geraedts JP, *et al*. Decreased human semen quality and organochlorine compounds in blood. *Hum Reprod* 2002;17: 1973–9.
- 51 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.
- 52 Goncharov A, Rej R, Negoita S, Schymura M, Santiago-Rivera A, *et al*. Akwesasne Task Force on the Environment. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in Native American men. *Environ Health Perspect* 2009;117: 1454–60.
- 53 Hagmar L, Bjork J, Sjodin A, Bergman A, Erfurth EM. Plasma levels of persistent organohalogen and hormone levels in adult male humans. *Arch Environ Health* 2001;56: 138–43.
- 54 De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, *et al*. Reduced seminal parameters associated with environmental DDT exposure and p, p'-DDE concentrations in men in Chiapas, Mexico: a cross-sectional study. *J Androl* 2006;27: 16–27.
- 55 Persky V, Turyk M, Anderson HA, Hanrahan LP, Falk C, *et al*. The effects of PCB exposure and fish consumption on endogenous hormones. *Environ Health Perspect* 2001;109: 1275–83.
- 56 Raymer JH, Michael LC, Studabaker WB, Olsen GW, Sloan CS, *et al*. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. *Reprod Toxicol* 2012;33: 419–27.
- 57 Specht IO, Hougaard KS, Spano M, Bizzaro D, Manicardi GC, *et al*. Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances—a study of spouses of pregnant women in three geographical regions. *Reprod Toxicol* 2012;33: 577–83.
- 58 Toft G, Jonsson BA, Lindh CH, Giwercman A, Spano M, *et al*. Exposure to perfluorinated compounds and human semen quality in arctic and European populations. *Hum Reprod* 2012; 27: 2532–40.
- 59 Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, *et al*. Do perfluoroalkyl compounds impair human semen quality? *Environ Health Perspect* 2009;117: 923–7.
- 60 Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, *et al*. An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. *J Occup Environ Med* 1998; 40: 614–22.
- 61 Akutsu K, Takatori S, Nozawa S, Yoshiike M, Nakazawa H, *et al*. Polybrominated diphenyl ethers in human serum and sperm quality. *Bull Environ Contam Toxicol* 2008; 80: 345–50.
- 62 Abdelouhab N, Ainmelk Y, Takser L. Polybrominated diphenyl ethers and sperm quality. *Reprod Toxicol* 2011; 31: 546–50.
- 63 Johnson PI, Stapleton HM, Mukherjee B, Hauser R, Meeker JD. Associations between brominated flame retardants in house dust and hormone levels in men. *Sci Total Environ* 2013;445–446: 177–84.
- 64 Meeker JD, Johnson PI, Camann D, Hauser R. Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. *Sci Total Environ* 2009;407: 3425–9.
- 65 Mocarelli P, Gerthoux PM, Patterson DG Jr, Milani S, Limonta G, *et al*. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. *Environ Health Perspect* 2008;116: 70–7.
- 66 Bhatia R, Shiao R, Petreas M, Weintraub JM, Farhang L, *et al*. Organochlorine pesticides and male genital anomalies in the child health and development studies. *Environ Health Perspect* 2005;113: 220–4.
- 67 Longnecker MP, Klebanoff MA, Brock JW, Zhou H, Gray KA, *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.
- 68 Rignell-Hydbom A, Lindh CH, Dillner J, Jonsson BA, Rylander L. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants and the risk of hypospadias. *PLoS One* 2012;7: e44767.
- 69 Virtanen HE, Koskeniemi JJ, Sundqvist E, Main KM, Kiviranta H, *et al*. Associations between congenital cryptorchidism in newborn boys and levels of dioxins and PCBs in placenta. *Int J Androl* 2012;35: 283–93.
- 70 McGlynn KA, Guo X, Graubard BI, Brock JW, Klebanoff MA, *et al*. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. *Environ Health Perspect* 2009;117: 1472–6.
- 71 Pierik FH, Klebanoff MA, Brock JW, Longnecker MP. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. *Environ Res* 2007;105: 364–9.
- 72 Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, *et al*. Cryptorchidism Study Group from Nice Area. Cryptorchidism at birth in Nice area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. *Hum Reprod* 2008;23: 1708–18.
- 73 Main KM, Kiviranta H, Virtanen HE, Sundqvist E, Tuomisto JT, *et al*. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. *Environ Health Perspect* 2007; 115: 1519–26.
- 74 Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, *et al*. Effects of environmental androgens on reproductive development in experimental animals. *Hum Reprod Update* 2001;7: 248–64.
- 75 Torres-Sanchez L, Zepeda M, Cebrían ME, Belkind-Gerson J, Garcia-Hernandez RM, *et al*. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. *Ann N Y Acad Sci* 2008; 1140: 155–62.
- 76 Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, *et al*. *In utero* exposure to the antiandrogen 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, Mexico. *Am J Epidemiol* 2007;165: 1015–22.
- 77 Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. *Lancet* 2000;356: 1240–1.
- 78 Mocarelli P, Gerthoux PM, Needham LL, Patterson DG Jr, Limonta G, *et al*. Perinatal exposure to low doses of dioxin can permanently impair human semen quality. *Environ Health Perspect* 2011;119: 713–8.
- 79 Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Kristensen SL, *et al*.

- Associations of in Utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. *Environ Health Perspect* 2013; 121: 453–8.
- 80 Daston GP, Cook JC, Kavlock RJ. Uncertainties for endocrine disrupters: our view on progress. *Toxicol Sci* 2003;74: 245–52.
- 81 Herman-Giddens ME. Recent data on pubertal milestones in United States children: the secular trend toward earlier development. *Int J Androl* 2006;29: 241–6.
- 82 Ong KK, Ahmed ML, Dunger DB. Lessons from large population studies on timing and tempo of puberty (secular trends and relation to body size): the European trend. *Mol Cell Endocrinol* 2006;254–255: 8–12.
- 83 Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, *et al*. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: sharpe and Skakkebaek's hypothesis revisited. *Environ Health Perspect* 2002;110: 771–6.
- 84 Grandjean P, Gronlund C, Kjaer IM, Jensen TK, Sørensen N, *et al*. Reproductive hormone profile and pubertal development in 14-year-old boys prenatally exposed to polychlorinated biphenyls. *Reprod Toxicol* 2012;34: 498–503.
- 85 Korrick SA, Lee MM, Williams PL, Sergeev O, Burns JS, *et al*. Dioxin exposure and age of pubertal onset among Russian boys. *Environ Health Perspect* 2011;19: 1339–44.
- 86 Lopez-Espinosa MJ, Fletcher T, Armstrong B, Genser B, Dhataria K, *et al*. Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant. *Environ Sci Technol* 2011;45: 8160–6.
- 87 Humblet O, Williams PL, Korrick SA, Sergeev O, Emond C, *et al*. Dioxin and polychlorinated biphenyl concentrations in mother's serum and the timing of pubertal onset in sons. *Epidemiology* 2011;22: 827–35.
- 88 Gladen BC, Klebanoff MA, Hediger ML, Katz SH, Barr DB, *et al*. Prenatal DDT exposure in relation to anthropometric and pubertal measures in adolescent males. *Environ Health Perspect* 2004;112: 1761–7.
- 89 Hass U, Boberg J, Christiansen S, Jacobsen PR, Vinggaard AM, *et al*. Adverse effects on sexual development in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. *Reprod Toxicol* 2012;34: 261–74.

How to cite this article: Vested A, Giwerzman A, Bonde JP, Toft G. Persistent organic pollutants and male reproductive health. *Asian J Androl* 2013 Dec 16. doi: 10.4103/1008-682X.122345. [Epub ahead of print]

