10 Epidemiologic Evidence on the Relationship Between Environmental Endocrine Disruptors and Male Reproductive and Developmental Health

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1. INTRODUCTION

There is scientific, governmental, and public concern over the potential adverse human health risks of exposure to environmental endocrine-disrupting chemicals (eEDCs). Human health endpoints of concern include, among others, (i) disrupted reproductive function, manifest as infertility or early pregnancy loss, (ii) altered fetal development, manifest as urogenital tract abnormalities in male newborns, including hypospadias and cryptorchidism, (iii) altered thyroid function, and (iv) increased risk of reproductive cancers including breast in women and testicular and prostate in men. Other endpoints that have also been studied include increased risk of endometriosis and altered birth sex ratios.

Given the wide range of potential health endpoints, it would not be possible to synthesize the existing epidemiologic evidence in a single chapter. Therefore, we made the decision to focus on a subset of literature, specifically male reproductive and developmental health endpoints, for which there is some human epidemiologic

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evidence. In addition, because it has been hypothesized that several of the male reproductive and developmental endpoints may be linked through a common casual pathway, testicular dysgenesis syndrome (TDS) (*1*), which may include exposure to environmental endocrine-disrupting chemicals (eEDCs), we welcomed the opportunity to explore their relationship with eEDCs in depth.

Our understanding of the mechanisms through which environmental chemicals alter the endocrine system is derived largely from experimental studies in laboratory animals and in vitro systems. In observational epidemiologic studies, it is generally not possible to explore potential mechanisms of the eEDCs. Nevertheless, epidemiologic studies are essential to our understanding of the potential risks, or lack thereof, of eEDCs on human reproductive function and development. Because this chapter is a synthesis of the epidemiologic literature on eEDCs and male reproductive function and development, experimental data on mechanisms of eEDCs are only briefly discussed. We refer readers to Chapter 3 of this book by Cowin, Foster, and Risbridger for further discussion of the basic biology of effects of eEDCs on male reproductive health. Environmental eEDCs can alter male reproductive function and development through multiple mechanisms. Two relevant mechanisms include anti-androgenic and estrogenic effects. Anti-androgenic chemicals can act directly as androgen receptor (AR) antagonists. This includes p,p'-DDE, the primary metabolite of DDT (*2*). Chemicals can also act as anti-androgens without directly interacting with the AR. For example, some phthalates can inhibit fetal testosterone synthesis by the fetal rat Leydig cells (*3*).

Chemicals that are estrogenic can, at high doses, alter male development by down-regulating ARs (*4*). This may lower androgen action and elevate estrogen action simultaneously, leading to an altered androgen–estrogen balance that may then alter reproductive tract development (*5*). Evidence for this has been seen in experimental studies with diethylstilbestrol (DES), a potent synthetic estrogen. However, weaker estrogens, such as bisphenol A (BPA), at environmentally relevant levels have not been shown to induce similar effects following neonatal exposure (*5*). These weaker estrogens at lower doses had positive effects on the rat testis and accelerated normal onset of pubertal spermatogenesis (*6*).

Polychlorinated biphenyls (PCBs) can alter endocrine function through estrogenic agonist or antagonist activities that may be at the level of the estrogen receptor or may influence biological effects of estradiol by altering its metabolism. For instance, PCB metabolites (OH-PCBs) may alter metabolism of endogenous estrogens by inhibiting sulfotransferases (SULT) (*7*). Furthermore, dioxin-like PCBs, as well as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), may induce activation of AhR, which induces the expression of CYP450 genes that are involved in estrogen metabolism (*8*). Studies have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in rats reduces plasma testosterone and dihydrotestosterone (DHT) levels by inhibiting steroidogenesis and the mobilization of cholesterol to CYP450scc (a mitochondrial enzyme that converts cholesterol into pregnenolone) (*9*). TCDD has also been shown to have anti-estrogenic effects by down-regulating ER (*10*).

Because androgens and estrogens play key roles in regulating reproduction and development, it is biologically plausible that environmental EDCs that possess anti-androgenic activity or estrogenic activity (either directly at the level of the receptor or indirectly through steroid synthesis or metabolism) may influence the human reproductive system. However, despite the experimental data on the endocrine-disrupting

mechanisms of eEDCs, human evidence of altered male reproductive and developmental health in relation to eEDCs is limited.

Furthermore, although well appreciated by the scientific community, the potential effect on human health from exposure to mixtures of eEDCs is not well studied. As has been shown in the recent Third Report by CDC (11), humans are exposed to at least hundreds of environmental chemicals, of which dozens are known EDCs. A major limitation of epidemiologic studies is that they generally only measure human exposure to a single EDC or at best to a set of isomers or congeners within a family of EDCs. A fuller understanding of potential human health risks will require studying the complex mixtures to which we are exposed. This limitation should be kept at the forefront as we discuss the current epidemiologic evidence on health risks from eEDCs.

2. MALE REPRODUCTIVE FUNCTION AND DEVELOPMENT

Some epidemiologic studies suggest that human semen quality has declined during the previous 50 years (12–14). Further evidence for this comes from a recent study among healthy young men that found an unexpectedly high proportion of poor semen quality (15). However, there are other epidemiologic studies that have not reported a decline in semen quality (16–18). Epidemiologic studies also suggest that there are temporal upward trends in the rates of testicular germ cell cancers (19–21) and male urogenital tract abnormalities (22), such as cryptorchidism and hypospadias.

Some scientists hypothesize that environmental exposures to low levels of EDCs may be partially responsible for these trends. Skakkebaek et al. (1) proposed that the trends in semen quality, testicular germ cell cancer, and male urogenital tract anomalies may share a common causal pathway and defined this triad as the TDS. The hypothesis invokes a common pathway through which EDCs, and other environmental chemicals and genetic factors, may lead to abnormal development of the fetal testis, producing testicular dysgenesis. This in turn can manifest as an increased risk of urogenital abnormalities in newborn males, as well as altered semen quality and testicular germ cell cancer in young men.

Despite the concern raised by the trends in male reproductive and developmental health endpoints, the epidemiologic data on their potential relationships with exposure to eEDCs remain limited and generally inconclusive. There are epidemiologic studies on the putative relationship between semen quality and adult exposure to three classes of eEDCs: PCBs, pesticides (persistent and non-persistent), and phthalates. In addition, there are also epidemiologic studies of the relationship between pesticides and phthalates and the prevalence of hypospadias and/or cryptorchidism in newborn males. Finally, a provocative recent study on the relationship between reduced anogenital distance among infant boys and exposure to phthalates will be discussed as a potential link between human exposure and alterations in urogenital tract development.

3. SEMEN QUALITY AND eEDCs

Although semen quality is measured in the adult male, it may be affected by EDC exposure during earlier life stages, such as during gestation or peri-pubertal development. In addition, as recently shown by Anway et al. (23), there are transgenerational effects of anti-androgenic chemicals, whereby exposure of the maternal or paternal (or even the grandparents) gametes to chemicals may confer an increased risk of altered

semen quality in the offspring. Although evidence from studies in laboratory animals and human studies on prenatal exposure of men to DES supports early life exposure effects on spermatogenesis, the epidemiologic evidence on the relationship between semen quality and exposure to eEDCs is limited to the assessment of adult exposure to eEDCs. The explanation for this is simply that studies on adult exposures are more straightforward to design and implement.

We specifically focus on epidemiologic studies that explored the relationship of semen quantity and quality with PCBs, DDT, non-persistent pesticides, and phthalates. The majority of the epidemiologic studies described are cross-sectional designs in which exposure and semen parameters were assessed at the same time, in the adult. One of the limitations of a cross-sectional design is that the onset and timing of exposure in relation to outcome is not always clear.

In the majority of studies, subject participation rates were generally very low, raising concern with selection bias *(24)*. Recently, we showed that selection bias in the setting of an infertility clinic was unlikely to be important *(25)*. Finally, most studies collected a single semen sample. Because of the known within individual variability in semen parameters, there may be misclassification of semen quality, which would generally tend to bias associations, if present, to the null hypothesis.

3.1. Phthalates and Semen Quality

The diesters of 1,2-benzenedicarboxylic acid (phthalic acid), commonly known as phthalates, are a group of man-made chemicals widely used in industrial applications. High-molecular weight phthalates [e.g.,di(2-ethylhexyl) phthalate (DEHP)], are primarily used as plasticizers in the manufacture of flexible vinyl plastic which, in turn, is used in consumer products, flooring and wall coverings, food contact applications, and medical devices *(26–28)*. Manufacturers use low-molecular weight phthalates [e.g., diethyl phthalate (DEP) and dibutyl phthalate (DBP)] in personal-care products (e.g., perfumes, lotions, cosmetics), as solvents and plasticizers for cellulose acetate, and in making lacquers, varnishes, and coatings, including those used to provide timed releases in some pharmaceuticals *(26,29,30)*.

As a result of the ubiquitous use of phthalates in personal-care and consumer products, human exposure is widespread. Exposure through ingestion, inhalation, and dermal contact are considered important routes of exposure for the general population *(27–32)*. Parenteral exposure from medical devices and products containing phthalates are important sources of high exposure to phthalates, primarily DEHP *(28,33)*. Upon exposure, phthalates are rapidly metabolized and excreted in urine and feces *(27–30)*. The most common biomonitoring approach for investigating human exposure to phthalates is the measurement of urinary concentrations of phthalate metabolites.

As compared with the laboratory animal data on the reproductive toxicity of phthalates, the human data are limited (Table 1). In an early study on phthalates and semen quality, Murature and coworkers *(34)* measured DBP concentrations in the cellular fractions of ejaculates from 21 university students and found an inverse relationship with sperm concentration. The study was small and did not adjust for potential confounders. In another small study, conducted in India, Rozati and coworkers *(35)* studied 21 infertile men with poor semen quality and 32 "control" men with normal semen parameters. Phthalate esters were measured in seminal plasma, and the results

Table 1
Summary Table of Epidemiologic Studies (in Chronological Order) on the Relationship Between Phthalates and Semen Quality

Author (country)	*Study population*	*Exposure*	*Results*	*Comments*
Murature et al., 1987 (34) (USA)	21 young men	DBP in cellular fractions of ejaculates	In men with "low ability to metabolize DBP," inverse relationship between sperm concentration and DBP ($r = -0.4$; slope of regression was -0.7). In the "men with a greater ability to metabolize DBP," there was also an inverse correlation of -0.4 (slope of regression -0.6) between DBP and sperm concentration	Small sample size, no adjustment for confounders, and measured diester
Rozati et al., 2002 (35) (India)	53 men (21 infertile and 32 controls)	Seminal plasma levels of phthalates (DBP, BBzP, DEHP, and DnOP)	Sum of phthalates was inversely correlated with sperm morphology ($r = -0.77$, $p < 0.001$) and positively correlated with the percentage of single-stranded DNA in sperm ($r = 0.86$, $p < 0.001$) assessed with the sperm nuclear chromatin condensation test. The concentration of phthalates was not correlated with ejaculate volume, sperm concentration, or motility	Measured total phthalate diesters concern with contamination
Duty et al., 2003 (36) (USA)	168 men from an infertility clinic (semen parameters)	Urinary levels of phthalate metabolites (MBP, MBzP, MEP, MEHP, and MMP)	Dose–response relationships (after adjusting for age, abstinence time, and smoking status) between MBP and sperm motility (OR per tertile = 1.0, 1.8, and 3.0; p for trend = 0.02) and sperm concentration (OR per tertile = 1.0, 1.4, and 5.5; p for trend = 0.07). Dose–response relationship between MBzP and sperm concentration (OR per tertile = 1.0, 1.4, and 5.5; p for trend = 0.02)	Confounders considered: age, BMI, abstinence time, smoking status, and race
Jonsson et al., 2005 (37) (Sweden)	234 young men	Urinary levels of MEP, MEHP, MBzP, MBP, and phthalic acid	No relationships of MBP, MBzP, or MEHP with any of the semen parameters. The highest quartile for MEP had fewer motile sperm (mean difference was 8.8%, 95% CI = 0.8, 17) and more immotile sperm (8.9%, 95% CI = 0.3–18). Phthalic acid was associated with improved function as measured by more motile sperm and fewer immotile sperm	Confounders considered: abstinence time and smoking status

BBzP, butylbenzyl phthalate; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DiNP, di-isononyl phthalate; DnOP, di-*n*-octyl phthalate; MBP, monobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, monoethylhexyl phthalate; and MEP, monoethyl phthalate.

were reported as the sum of a mixture of dimethyl phthalate, DEP, DBP, butylbenzyl phthalate (BBzP), DEHP, and di-*n*-octyl phthalate. The concentration of phthalates was inversely correlated with sperm morphology but not correlated with ejaculate volume, sperm concentration, or motility. In this study, as in the Murature study, the measurement of phthalate diesters raises concern with sample contamination from the ubiquitous presence of the diester in the environment.

More recently, a larger study using urinary levels of phthalate metabolites was conducted by Duty and colleagues (*36*). Study subjects consisted of male partners of subfertile couples who presented to an infertility clinic in Massachusetts, USA. At the time of the clinic visit, one sample of semen and of urine were collected from 168 men. There were dose–response relationships (after adjusting for age, abstinence time, and smoking status) between monobutyl phthalate (MBP, the hydrolytic metabolite of DBP) and below World Health Organization (WHO) reference value sperm motility and sperm concentration (*36*). There was also a dose–response relationship between monobenzyl phthalate (MBzP, the primary hydrolytic metabolite of BBzP) and below WHO reference value sperm concentration.

In a recently published study from Sweden, Jonsson and colleagues (*37*) recruited 234 young Swedish men at the time of their medical conscript examination. Each man provided a single urine sample used to measure concentrations of MEP, MEHP, MBzP, MBP and phthalic acid.

In contrast to the US study, in the Swedish study there were no relationships of MBP or MBzP with any of the semen parameters. MEHP was also not associated with any of the semen parameters. Men in the highest quartile for MEP had fewer motile sperm and more immotile sperm than men in the lowest MEP quartile. Contrary to their hypothesis, phthalic acid was associated with improved function as measured by more motile sperm and fewer immotile sperm. Phthalic acid is a non-specific marker of phthalate exposure, formed as the result of the hydrolysis of any of the phthalates measured. Interactions between urinary phthalate levels and PCB 153 (measured previously in serum samples from these men) were assessed by including an interaction term in the models. There was no evidence of multiplicative interactions between PCB 153 and any of the phthalates with the reproductive markers (data was not shown). This is in contrast to a previous study by Hauser et al. (*38*), who reported interactions of MBP and MBzP with congener PCB 153 in relation to sperm motility.

Although the Swedish study had some similarities to the US study, as they were both cross-sectional studies in which a single urine and semen sample were collected, there were many important differences. One of the primary differences was in the age of the study population and the method of recruitment. The Swedish study population consisted of young men (median age 18 years, range 18–21 years) undergoing a medical examination before military service. As approximately 95% of young men in Sweden undergo the conscript examination, these young men reflected the general population of young Swedish males. In contrast, in the US study, the median age of the men recruited from an infertility clinic was 35.5 years and ranged from 22 to 54 years. None of the men from the infertility clinic were 21 years of age or younger. The differences across studies in the ages and source of the men may account for some of the differences in the results between studies. For instance, it is unclear whether men presenting to an infertility clinic are more "susceptible" to reproductive toxicants, including phthalates, than men from the general population. Furthermore, it is also unclear

whether middle-aged men, as compared with young men, are more "susceptible" to reproductive toxicants because of an age-related response to the toxicant.

Other differences across studies include major differences in participation rates (14% in the Swedish study and 65% in the US study). This is unlikely to introduce selection bias, because the Swedish young men would have to participate differentially in relation to both reproductive function and phthalate levels, which is unlikely as they did not know their phthalate exposure or semen quality status. Another difference across studies is differences in the analytical methods used to measure urinary phthalate metabolites. The higher limits of detection and lower analytical precision in the Swedish study may have contributed to measurement error of urinary phthalate levels and may result in bias to the null hypothesis. However, by categorizing the phthalate levels into quartiles for the statistical analysis, this would minimize concern with measurement error resulting from the analytical imprecision and low detection limits.

In summary, the epidemiologic data on the relationship between semen quality and phthalate exposure remains limited and inconsistent. Although the two recent studies by Duty et al. *(36)* and Jonsson et al. *(37)* had some similarities, important differences existed. Additional studies are critically needed to help elucidate possible explanations for differences across studies and most importantly to address whether phthalate exposure alters semen quality.

3.1.1. Semen Quality and Environmental PCB Exposure

PCBs are a class of synthetic, persistent, lipophilic, halogenated aromatic compounds that were widely used in industrial and consumer products for decades before their production was banned in the late 1970s. PCBs were used in cutting oils, lubricants, and as electrical insulators. As a result of their extensive use and persistence, PCBs remain ubiquitous environmental contaminants. They are distributed worldwide and have been measured in air, water, aquatic, and marine sediments, fish, and wildlife *(39)*. Furthermore, they are biologically concentrated and stored in human adipose tissue. The general population is exposed primarily through ingestion of contaminated foods (e.g., fish, meat, and dairy products), as PCBs can bioaccumulate up the food chain. However, exposure may also occur through dermal contact (soil and house dust) and inhalation (indoor air in residential buildings and workplaces, as well as outdoor air). For example, in the 1960s and 1970s, PCBs were used in sealants for commercial building construction, and high levels of PCBs (up to 36, 000 ppm) have been found to remain in the caulking of some public buildings, which may lead to contamination of indoor air and dust *(40)*. As a result of their persistence and ubiquity, measurable levels of serum PCBs are found in the majority of the US general population *(41)*. Serum levels of PCBs are an integrated measure of internal dose, reflecting exposure from all sources over the previous years; depending on the congener, the half-life of PCBs in the blood ranges from 1 to 10 or more years *(42,43)*.

In one of the early studies on environmental exposure to PCBs and semen quality, Bush and coworkers *(44)* collected semen samples from fertile men ($n = 33$), men with oligozoospermia ($n = 50$) or azoospermia ($n = 50$) and men status postvasectomy ($n = 25$) (Table 2). The seminal plasma levels of the PCB congeners 153, 138, and 118 were inversely related to sperm motility but only among semen samples with a sperm count <20 million/ml. Because an association was only found among a subset of the subjects, caution should be used to interpret these results.

Table 2
Summary Table of Epidemiologic Studies (in Chronological Order) on the Relationship of Polychlorinated Biphenyls and *p,p*-DDE with Semen Quality

Author	*Study population*	*Exposure*	*Results*	*Comments*
Bush et al., 1986 (44)	33 fertile, 50 subfertile, 50 infertile, and 25 post-vasectomy men	Seminal plasma levels of PCBs and p,p'-DDE	PCB 153, 138, and 118 were inversely related to sperm motility only among samples with a sperm count <20 million/ml. No associations of semen parameters with p,p'-DDE.	Association found only among a subset of men
Rozati et al., 2002 (35)	53 men from India (21 infertile and 32 controls)	Seminal plasma levels of PCBs	PCBs detected in the seminal plasma of infertile men but not controls. Negative correlation between PCBs and total progressive motility ($r = -0.5$) and positive correlation with percentage of single-stranded DNA in sperm ($r = 0.6$). No correlations with sperm count, rapid progressive motility, or normal morphology	Data on individual PCB congeners not presented. No statistical adjustment for potential confounders
Dallinga et al., 2002 (45)	65 Dutch men from an infertility clinic	Serum and semen levels of PCB 118, 138, 153, 180, and their metabolites	Seminal plasma PCB levels among men with good semen quality were higher than among men with poor semen quality (0.071 ng/ml and 0.022 ng/ml, respectively, $p = 0.06$). In men with good semen quality, there were inverse associations between serum levels of sum of PCB metabolites and sperm count ($p = 0.04$) and progressive motile sperm concentration ($p = 0.02$). There were also negative non-significant corresponding associations in men with poor semen quality	Confounders considered: age and smoking status. Measured PCB metabolites
Richthoff et al., 2003 (46)	305 Swedish young men	Serum levels of PCB 153	Inverse association between PCB 153 and percent motile sperm (10 ng/g lipid increase in PCB 153 associated with a 1.0% decline in percent CASA motile sperm (95% CI = −2.0 to −0.13)]. No association of PCB 153 with sperm concentration	Confounders considered: BMI, abstinence period, and smoking status
Hauser et al., 2003 (48)	212 US men from an infertility clinic	Serum levels of PCBs and p,p'-DDE	Dose–response relationships (odds ratio per tertile adjusted for age, abstinence time, and smoking status) between PCB 138 and below reference sperm motility (1.00, 1.68, and 2.35, respectively; p-value for trend is 0.04) and sperm morphology (1.00, 1.36, and 2.53; $p = 0.04$). DDE had a non-significant association with sperm motility	Confounders considered: BMI, age, abstinence period, and smoking status

Rignell-Hydbom et al., 2004 (47)	195 Swedish fishermen	Serum levels of PCB 153 and p,p'-DDE	The highest PCB 153 quintile had decreased sperm motility as compared with men in the lowest quintile. The age adjusted mean difference was 9.9% (95% CI = −1.0 to − 21%, $p = 0.08$). No significant associations of p,p'-DDE with semen parameters	Confounders considered: age, smoking status, abstinence time, BMI, and reproductive hormones
High-exposure studies				
Guo et al., 2000 (50)	35 young men from Taiwan (12 prenatally exposed to contaminated rice oil and 23 unexposed men)	Maternal ingestion (yes/no) of rice oil contaminated with PCBs and PCDFs	Increased abnormal morphology in exposed men (37.5%) as compared with unexposed men (25.9%). Exposed men had decreased percentage of motile sperm (35.1% compared with 57.1% in unexposed men) and rapidly motile sperm (25.5% compared with 42.4% in unexposed men). Reduced hamster oocyte penetration in exposed men	Age and percent smokers in exposed and unexposed groups were similar. No statistical adjustment for confounders
Hsu et al., 2003 (51)	68 men from Taiwan (40 exposed to contaminated rice oil and 28 unexposed)	Ingestion (yes/no) of rice oil contaminated with PCBs and PCDFs	Exposed men had higher percentage of sperm with abnormal morphology (27.5%) compared with unexposed men (23.3%) and a higher oligospermia rate (9% compared with 1%, respectively). Ability of sperm to penetrate the hamster oocyte was reduced in exposed men	Age and percent smokers in exposed and unexposed groups were similar. No statistical adjustment for confounders

BMI, body mass index; PCBs, polychlorinated biphenyls; and PCDFs, polychlorinated dibenzofurans.

In a more recent study in the Netherlands, Dallinga and colleagues (*45*) studied men who were partners in couples visiting an infertility treatment center. Based on progressive motile sperm concentration, they identified a group of men with good semen quality ($n = 31$) and a group of men with very poor semen quality ($n = 34$). Blood and semen were analyzed for PCB 118, 138, 153, and 180 and their hydroxylated metabolites. Contrary to expectations, the sum of PCBs in seminal plasma of men with good semen quality were higher than among men with poor semen quality ($p = 0.06$). However, within the group of men with good semen quality, there were inverse associations between serum levels of sum of PCB metabolites and sperm count ($p = 0.04$) and progressive motile sperm concentration ($p = 0.02$). There were also inverse non-significant corresponding associations in the men with poor semen quality. Because associations with semen quality were found for PCB metabolites and not the parent PCBs, these results suggested that the PCB metabolites were the biologically active compounds.

In Sweden, Richthoff and coworkers (*46*) studied 305 young men 18–21 years of age undergoing a conscript examination for military service. There were significant inverse associations between PCB 153, a good biomarker of exposure to total PCBs, and percent motile sperm. There were no associations between PCB 153 and sperm concentration or total sperm count. Although the participation rate was very low, only 13.5% of eligible subjects agreed to participate, it is unlikely that this would introduce bias as young men are likely to be unaware of their fertility or exposure levels.

In India, Rozati and coworkers (*35*) studied infertile men and controls (study details are provided Section 3.1.). They reported a negative correlation between seminal plasma PCB levels and total progressive motility and a positive correlation with percentage of single-stranded DNA in sperm. No correlations were found between PCBs and sperm count, rapid progressive motility, or normal morphology. The authors reported results for total PCBs and not for individual congeners. Potential confounders were considered in the methods section, but no adjustments were made in the analysis.

Rignell-Hydbom et al. (*47*) studied 195 Swedish fishermen (median age 50.6 years, ranged from 24–65 years) from the east and west coasts. The highest PCB 153 quintile had decreased sperm motility compared with men in the lowest quintile. There were no consistent associations of PCB 153 with sperm concentration. Although p,p'-DDE was inversely associated with sperm motility, when age was included in the models, the association became weaker and non-significant.

In the US, Hauser and colleagues (*48*) studied 212 male (mean age was 36.0 years) partners of subfertile couples visiting an infertility clinic. Fifty-seven PCB congeners including PCB 118, 138, 153, and 180 were measured. There were significant dose–response relationships (increasing odds ratios with increasing PCB tertile adjusted for age, abstinence time, and smoking status) between PCB 138 and below WHO reference sperm motility and sperm morphology. Associations between semen parameters and PCB 153 were not consistent. p,p'-DDE showed a weak non-significant relationship with sperm motility.

Ayotte and coworkers (*49*) reported on the association between p,p'-DDE, a major biologically persistent metabolite of DDT, and semen quality in 24 young men from Chiapas, Mexico. The men, 16–28 years of age, were non-occupationally exposed to DDT. The mean concentration of p,p'-DDE was several hundred fold higher than levels in men from other countries, such as the US and Canada, where DDT was not

recently used. p,p'-DDE was inversely correlated with both semen volume and sperm count. Although the study was small and did not control for potential confounders, the results are intriguing and worthy of replication in other cohorts.

3.1.2. Semen Quality and High PCB Exposure

Guo and colleagues *(50)* studied the relationship between semen quality and prenatal exposure to PCBs and PCDFs after the poisoning episode in Taiwan in 1979 in which PCB-contaminated rice oil was ingested. Twelve men with prenatal exposure to contaminated rice oil and 23 healthy unexposed subjects of comparable age provided a semen sample. The unexposed men had no unusual chemical exposure and were recruited from a local high school. The mean (SD) age of the exposed men was 17.3 (1.2) years and 17.6 (1.0) for the unexposed men. The proportion of sperm with abnormal morphology was increased in the exposed men. In the exposed men, the percentage of motile sperm and rapidly motile sperm were reduced. In addition, sperm from exposed men had reduced hamster oocyte penetration as compared with unexposed men. This small study provided the opportunity to explore high prenatal exposure to PCBs and PCDFs.

In a second study on men from the Taiwan PCB poisoning episode, Hsu and coworkers *(51)* studied the relationship between semen quality and levels of PCBs among men that consumed contaminated rice oil some 20 years earlier. They identified 40 exposed men and 28 unexposed men. Mean age of exposed (37.9 years) and unexposed (40.4) were similar. Exposed men had a higher percentage of sperm with abnormal morphology and a higher oligozoospermia rate. The ability of sperm to penetrate the hamster oocyte was reduced in exposed men. The results of this study provide evidence of adverse effects of exposure to PCBs and PCDFs among men exposed 20 years earlier to the contaminated rice oil.

The data on the relationship between PCBs and semen quality support an inverse association of PCBs with reduced semen quality, specifically reduced sperm motility. The associations found were generally consistent across studies performed in different countries (India, Netherlands, Taiwan, Sweden, and USA) that used different methods to measure semen quality and PCBs. Furthermore, associations were consistently found despite a range of PCB levels, that is there did not appear to be a threshold. The PCB levels in these studies ranged from low-background levels *(43,46,48)* to high-background levels due to consumption of contaminated fish *(47)*, and to even higher exposure levels due to ingestion of contaminated rice oil *(50,51)*. Although the data across studies generally support a relationship between PCBs and poor semen quality, there are possible alternative explanations. One potential alternative explanation is that PCBs are a surrogate for exposure to other environmental factors that may predict semen quality. Although this is possible, there is currently no evidence identifying potential alternative exposures. Another explanation is that there may be confounding of the associations by some currently unrecognized or unmeasured confounders. However, the more recent studies considered important potential confounders, and the results were consistent across studies. In conclusion, although PCBs are no longer used, these data, along with ongoing human exposure, albeit at lower levels than several decades ago, raise concerns regarding altered human fertility due to adverse affects on semen quality.

3.2. Semen Quality and Non-persistent Pesticides

Non-persistent pesticides (also referred to as "contemporary-use pesticides") are chemical mixtures that are currently available for application to control insects (insecticides), weeds (herbicides), fungi (fungicides), or other pests (e.g., rodenticides), as opposed to pesticides that have been banned from use in most countries (e.g., many of the formerly popular organochlorine pesticides such as DDT). Three common classes of non-persistent pesticides in use today include organophosphates, carbamates, and pyrethroids. Though environmentally non-persistent, owing to the extensive use of pest control in these various settings, a majority of the general population is exposed to some of the more widely used pesticides at low levels. Exposure among the general population occurs primarily through the ingestion of foods that contain low levels of pesticide residue or through inhalation and/or dermal exposure in or around the home and in other indoor environments. Non-persistent pesticides that are applied indoors or tracked in from outdoors may persist for extended periods while protected from sunlight, rain, temperature extremes, and most microbial action (*52*). For example, chlorpyrifos, a semivolatile organophosphate insecticide the use of which was recently restricted in the USA by the US Environmental Protection Agency, was measured in indoor air 4 years after pest control application in a home (*53*).

There are several epidemiologic studies on men exposed to non-persistent pesticides during agricultural work (Table 3). A cross-sectional study on testicular function measured sperm concentration, motility, and morphology in 122 greenhouse workers defined as low, medium, or highly exposed to more than a dozen pesticides (*54*). Adjusting for abstinence time and other potential confounders, a higher proportion of abnormal sperm was found in the high exposure group compared with the group with low exposure. Lower median sperm concentration was also observed in workers with more than 10 years of work in the greenhouse compared with men with less than 5 years of experience. In a cross-sectional study on traditional and organic farmers, Juhler and coworkers (*55*) investigated the relationship between dietary exposure to pesticides and semen quality. Estimating exposure through food frequency questionnaires and data from pesticide monitoring programs, the authors found that men with a lower intake of organic food had a lower proportion of normal-shaped sperm according to the strict criteria (2.5 vs. 3.7%, $p = 0.003$). However, organic food intake was not associated with the other 14 semen parameters measured in the study. Results in the study were adjusted for age, urogenital disease, spillage, abstinence time, smoking, and alcohol intake. Oliva and coworkers (*56*) recently investigated the impact of environmental factors on infertility among 177 men in Argentina. Adjusting for age, body mass index (BMI), abstinence time, income, health center, and smoking, a dose-related response was observed in (primary) infertile men occupationally exposed to pesticides. Significantly elevated odds ratios were reported for sperm concentration ($< 1 \times 10^6$/ml, OR = 3.4, 95% CI = 1.2 – 7.4), motility (<50% motile, OR = 3.6, 95% CI = 1.1 – 11.4), and morphology (<30% normal, OR = 4.1, 95% CI = 1.4 – 12.0) for men exposed to pesticides compared with occupationally non-exposed men. Conversely, in models adjusting for many of the same variables, Larsen and coworkers (*57*) found only marginal differences among 15 semen quality parameters from Danish farmers who sprayed pesticides compared with farmers who did not spray pesticides. These studies show a possible association between pesticide exposure and human semen

Table 3
Summary Table of Epidemiologic Studies (in Chronological Order) on the Relationship Between Non-Persistent Pesticides and Semen Quality

Author	*Study population*	*Exposure*	*Results*	*Comments*
Whorton et al., 1979 (61)	47 US carbaryl production workers plus 90 unexposed controls	Subjective exposure classification based on job tasks	Greater proportion of oligozoospermic men among the carbaryl workers (15%) as compared with the chemical workers (5.5%, $p = 0.07$)	No adjustment for potential confounders. Sperm motility not measured
Wyrobek et al., 1981 (62)	50 US carbaryl production workers plus 34 unexposed controls	Exposure ranks/groups based on job type held for previous year	Elevated percent of abnormal sperm in carbaryl workers (52%) as compared with comparison subjects (42%, $p < 0.005$). The proportion of men defined as teratospermics (>60% abnormal sperm) was higher among the carbaryl workers (28.6%) than in the comparison group (11.8%, $p = 0.06$)	Confounders considered: smoking, medical history, and previous exposure to hazardous agents
Padungtod et al., 2000 (58)	43 pesticide factory workers in China; 20 high exposed and 23 with no or very low exposure	Occupational exposure to ethyl parathion and methami-dophos	Exposure associated with reduction in sperm concentration and motility but not sperm morphology. Adjusted means for exposed and non-exposed workers were 28.5 and 49.4 million sperm/ml ($p = 0.01$), respectively, for sperm concentration and 64 and 74% ($p = 0.03$), respectively, for percentage of motile sperm	Confounders considered: age, abstinence period, and current smoking status
Swan et al., 2003 (64)	86 male partners from fertile couples attending US prenatal clinics	Urinary levels of pesticides or metabolites (IMPY, 1N, TCPY, and others)	Increased odds ratios (95% CI) for below reference semen parameters associated with high exposure group for alachlor mercapturate 30.0 (4.3–210), IMPY 16.7 (2.8–98), atrazine mercapturate 11.3 (1.3–99), 1-naphthol 2.7 (0.2–34), and TCPY 6.4 (0.5–86)	Small study size limited statistical power; odds ratios were unadjusted for potential confounders

(*Continued*)

Table 3
(*Continued*)

Author	*Study population*	*Exposure*	*Results*	*Comments*
Meeker et al., 2004 (65)	272 male partners from couples attending US infertility clinic	Urinary levels of insecticide metabolites (1N and TCPY)	Inverse association between urinary carbaryl metabolite (1N) and sperm concentration and motility. IQR increase in 1N associated with 16% decline in sperm concentration and 3.8% decline in motile sperm. Suggestive inverse association between chlorpyrifos metabolite (TCPY) and sperm motility	Confounders considered: age, BMI, abstinence time, smoking status, race, and season
Tan et al., 2005, 2006 (60,63)	31 workers in a Chinese pesticide factory exposed to carbaryl and fenvalerate; 46 internal and 22 external control subjects	Men defined as exposed or unexposed based on job tasks, air, and dermal monitoring	In exposed workers, seminal volume and sperm motility were lower than in the control groups ($p < 0.05$) as were the following sperm motion parameters: linearity, straightness, straight-line velocity, and beat cross frequency ($p < 0.05$)	Reported risk factors between groups were similar (health, age, smoking, and alcohol) but not included in the models

1N, 1-naphthol; BMI, body mass index; IMPY, 2-isopropoxy-4-methyl-pyrimidinol; and TCPY, 3,5,6-trichloro-2-pyridinol.

quality. However, the broad nature of the exposure assessments makes it impossible to determine which pesticides, if any, were responsible for the observed effects.

Few studies have been conducted that provide information on specific chemicals or classes of contemporary-use insecticides and altered testicular function. Padungtod and coworkers *(58)* studied the relationship between occupational exposure to organophosphates (parathion and methamidophos) and testicular function among Chinese pesticide factory workers. They found a significant reduction in adjusted mean sperm concentration (28.5 vs. 49.4 million sperm/ml, $p = 0.01$), and percentage of motile sperm (64 vs. 74%, $p = 0.03$) in the 20 exposed workers as compared with the 23 unexposed workers. In a recent Japanese study, pesticide sprayers exposed primarily to organophosphates and pyrethroids showed spraying season-dependant reductions in motile sperm velocity measures compared with unexposed controls *(59)*. Another recent study of 32 men occupationally exposed to the synthetic pyrethroid fenvalerate in a Chinese pesticide factory found that the exposed workers had decreased sperm counts, as well as declined sperm movement and progression, compared with men in both internal and external comparison groups *(60)*.

Two publications reported the results from a study on a small cohort of men exposed to the insecticide carbaryl (1-naphthyl methyl carbamate, commonly known as Sevin®) during the production and packaging of the compound *(61,62)*. Although analyses using sperm counts as a continuous measure failed to find significant differences based on carbaryl exposure, the authors found a greater proportion of oligozoospermic men among the carbaryl workers as compared with the chemical workers *(61)*. In a subsequent publication on the same cohort of carbaryl production workers, Wyrobek and coworkers *(62)* studied the relationship between sperm shape abnormalities and carbaryl. Morphological analyses showed an elevated percent of abnormal sperm in carbaryl workers as compared with comparison subjects, which remained after stratifying on potential confounders such as smoking, medical history, or previous exposure to hazardous agents. The proportion of men defined as teratospermics (>60% abnormal sperm) was higher among the carbaryl workers than in the comparison group (28.6 and 11.8%, respectively). More recently, a Chinese study of 31 carbaryl-exposed workers in a pesticide factory found significantly lower sperm motility and sperm motion parameters among the exposed men compared with men in internal and external comparison groups *(63)*.

Researchers are now utilizing urinary and serum biomarkers of pesticide exposure to explore associations with reduced semen quality. In a US study on the male partners of pregnant women, Swan and coworkers *(64)* compared urinary levels of pesticide biomarkers in 34 men with sperm concentration, motility, and morphology below the median (defined as cases) with 52 men with above median semen parameters (defined as controls). They found elevated odds ratios [OR (95% confidence interval)] for alachlor mercapturate [30.0 (4.3–210)], 2-isopropoxy-4-methyl-pyrimidinol (IMPY, diazinon metabolite) [16.7 (2.8–98)], atrazine mercapturate [11.3 (1.3–99)], 1-naphthol (carbaryl and naphthalene metabolite) [2.7 (0.2–34)] and 3,5,6-trichloro-2-pyridinol (TCPY, chlorpyrifos metabolite) [6.4 (0.5–86)]. However, a small study size led to the wide confidence intervals that restrict interpretation of the study results.

Also using urinary biomarker data representative of low environmental levels of pesticides commonly encountered among the general population, Meeker et al. *(65)* studied 272 men who were partners of an infertile couple. They found inverse

associations between urinary levels of 1-naphthol, a metabolite of both carbaryl and naphthalene, and sperm concentration and motility. They also found a suggestive inverse relationship between the urinary metabolite of chlorpyrifos and sperm motility. When insecticide metabolite levels were categorized into tertiles, odds ratios (95% CI) for medium and high tertiles of 1-napthol were 4.2 (1.4–13.0) and 4.2 (1.4–12.6) compared with the lowest tertile for below reference concentration (<20 million sperm/ml), and 2.5 (1.3–4.7) and 2.4 (1.2–4.5) for below reference motility (<50% motile sperm). In multiple linear regression analyses, an interquartile range increase in 1N (1.8–5.0 μg/l) was associated with a 3.9% (95% CI = −7.3 to −0.5%) decline in motile sperm and a 16% (−29 to +1.0%) decline in sperm concentration. An interquartile range increase in chlorpyrifos metabolite (TCPY, also 1.8–5.0 μg/l) was suggestively associated with a 2.2% (−5.1 to +0.7%) decline in motile sperm. In two follow-up studies conducted among an overlapping group of the same men, both TCPY and 1N were associated with declined serum testosterone levels *(66)*, whereas TCPY was also associated with decreased free thyroxine (T_4) and increased thyroid-stimulating hormone (TSH) *(67)*.

In summary, there are human data supporting an association between non-persistent pesticide exposure and altered semen quality though it is somewhat limited. Although the human data are suggestive, they are mostly derived from occupational studies involving simultaneous exposure to several pesticides. The relationship between reproductive health and exposure to specific non-persistent pesticides, and/or low-level mixed exposures through diet and residential use among the general male population, is not well understood. Additional research using biomarkers of exposure to specific pesticides is needed to further our understanding of the potential reproductive health risks associated with non-persistent pesticides.

4. CRYPTORCHIDISM, HYPOSPADIAS, AND eEDCs

4.1. Rationale for Linkage of Genital Anomalies to eEDC Exposure

The published literature addressing the possible role of eEDCs in the etiology of two common male genital anomalies, cryptorchidism, and hypospadias has grown rapidly in recent years. Several factors contributing to increasing levels of concern about the etiologic basis of these anomalies include (i) their induction by certain classes of eEDCs in rodents after prenatal exposure; (ii) their clinical correlation with risk of subfertility and testicular malignancy, other conditions that have been hypothesized to be linked to eEDCs; and (iii) reports of their increasing frequency in defined populations. The significance of each of these observations will be discussed in turn, followed by specific epidemiologic data regarding the association of specific eEDCs with cryptorchidism or hypospadias in human populations (Table 4).

Several classes of endocrine-disrupting chemicals including phthalates, pesticides and polychlorinated aromatic compounds are reproductive toxicants that can inhibit genital development and testicular descent in male rat offspring after maternal exposure *(68,69)* (see also Chapter 3 of this book by Cowin, Foster, and Risbridger). The mechanisms of action of these agents include but may not be limited to inhibition of AR activation, androgen synthesis inhibition, estrogenic effects, and/or inhibition of insl3 expression, effects that may be strain specific. Testosterone and its metabolite,

Table 4
Summary Table of Epidemiologic Studies (in Chronological Order) of the Relationship Between Pesticides and Cryptorchidism and/or Hypospadias

Author (country)	*Study population*	*Exposure*	*Results*	*Comments*
Longnecker et al., 2002 (94) (USA)	Birth cohort study 1959–1966; 22,347 boys, 219 with cryptorchidism, 199 with hypospadias, and 552 controls	Maternal serum levels of DDE during pregnancy	Adjusted OR of highest level of serum DDE with risk for: cryptorchidism, 1.2 (95% CI = 0.6–2.4) and hypospadias, 1.3 (0.7–2.4)	No consistent association found
Bhatia et al, 2005 (95) (San Francisco)	Birth cohort study 1959–1967; 9345 males followed until age 5, 101 with cryptorchidism, 73 with hypospadias, and 6 with both	Maternal serum levels of DDT and DDE during pregnancy	Adjusted OR of highest serum level of DDT with risk for cryptorchidism, 1.01 (0.44–2.28) and hypospadias, 0.79 (0.33–1.89). Adjusted OR of highest serum level of DDT with risk for cryptorchidism, 1.34 (0.51–3.48) and hypospadias, 1.18 (0.46–3.02)	No association of DDT or DDE with either anomaly
Garry et al., 1996 (96) (USA)	4935 births, 1989–1992, 20 cases of urogenital anomalies	34,772 parental pesticide appliers	Age adjusted OR for urogenital anomalies 1.89 (95% CI = 1.06–2.64)	Birth registry data. Authors did not explore associations separately for cryptorchidism and hypospadias
Garcia-Rodriquez et al., 1996 (97) (Spain)	270 cases of orchidopexy for cryptorchidism, 1980–1991	Predicted pesticide exposure level based on a four-point agricultural density scale	Logistic regression analysis of orchidopexy rates per 10,000 males aged 1–16: Rates increased with pesticide use (maximum 5.74 in highest pesticide use region) but highest rate (7.13) in urban low pesticide use region	Highest rate in urban area not consistent with relationship to pesticide exposure

(*Continued*)

Table 4
(*Continued*)

Author (country)	*Study population*	*Exposure*	*Results*	*Comments*
Kristensen et al., 1997 (98) (Norway)	192,417 births, 77 cases of cryptorchidism, and 70 cases of hypospadias	Parental occupation as farmer 1967–1991	Overall adjusted OR for cryptorchidism, 0.77 (0.58–1.03) and hypospadias, 1.00 (0.75–1.34); OR for pesticide purchase/cryptorchidism, 1.70 (1.16–2.5) and tractor spraying/hypospadias, 1.38 (0.95–1.99)	Confounders considered: year of birth, maternal age, region, and parental consanguinity
Weidner et al., 1998 (99) (Denmark)	Surgical records, 4226 cases of cryptorchidism, and 1345 cases of hypospadias	Maternal occupation as farmer or gardener 1983–1992	Risk of cryptorchidism: OR = 1.36 (95% CI = 1.1–1.73) for maternal farming or gardening and OR = 1.67 (95% CI = 1.14–2.47) for gardening only	No increased risk for paternal farming or gardening for hypospadias
Pierik et al, 2005 (100) (Netherlands)	8,698 births; 78 cases of cryptorchidism and 56 cases of hypospadias	Parental questionnaire data: self-reported and occupational exposures	Probable paternal exposure to pesticides, OR = 3.8 (95% CI = 1.1–13.4) for cryptorchidism	No association of pesticide exposure with hypospadias
Carbone et al, 2006 (101) (Sicily)	8,199 births; 59 cases of cryptorchidism and 54 cases of hypospadias	Geographical "pesticide impact" score based on use of spraying equipment and farm activity	Progressive increase in prevalence of hypospadias ($p = 0.003$, test for trend) or either anomaly ($p = 0.001$) but not cryptorchidism alone ($p = 0.096$) in relation to "pesticide" impact of geographical location	Registry data and review of all pediatric records, 1998–2002

DHT, are essential (particularly DHT) for masculinization of the genitalia but participate to a lesser degree in the process of testicular descent. Insl3 is critical for development of the gubernaculum, a fibromuscular fetal structure that guides the testis to a scrotal position. In rats, inhibition of nipple development and shortening of anogenital distance are the most sensitive markers of anti-androgenic effect (70). By contrast, phthalates, particularly DBP, are potent in inhibiting testicular descent, an effect that may be related to specific inhibition of insl3 expression in addition to inhibition of fetal testosterone production (71). However, the effective exposure levels are markedly higher than those estimated for the general human population (72). Although of concern, the applicability of these animal studies to human disease remains unclear, not only because experimental exposures are extremely high, but also because the genetic susceptibility of rats to cryptorchidism and hypospadias appears to be extremely low, unlike that of larger mammals and man.

In human males, the risk of non-syndromic cryptorchidism in full-term newborn males is 2–4% in most series (73) compared with a prevalence of hypospadias of 0.3–0.4%; 4–13% of males with hypospadias also have cryptorchidism (74). Cryptorchid males are at approximately 10 times increased risk for testicular germ cell cancer compared with the general population although the overall risk remains well below 1% (75). Reported paternity rates in previously unilaterally and bilaterally cryptorchid men are 90 and 65%, respectively (76). In view of the associations amongst these conditions, Skakkebæk et al. (1) hypothesized that a primary testicular abnormality, defined as "testicular dysgenesis syndrome" and possibly induced by eEDCs, is the common link between testicular germ cell tumors, reduced semen quality, and other reproductive tract abnormalities. They propose that TDS is due to prenatal Leydig and Sertoli cell dysfunction with secondary androgen insufficiency and impaired germ cell development. This entity should not be confused with the well-defined clinical entity known as dysgenetic testes, which is associated with genital ambiguity and a high risk of testicular malignancy (77). Reductions in the normal postnatal surge in androgen and LH production (78) and in germ cell maturation (79) in cryptorchidism provide support for a TDS-like global testicular dysfunction; however, other studies suggest that these findings are absent or less common than initially reported (75,80–82). Similarly, hypospadias alone has not been defined as a clear separate risk factor for infertility (74) or testicular cancer. The existence of TDS as a distinct clinical entity and a possible association with eEDCs, if present, have yet to be defined.

Recent trends over relatively short time periods showing increases in the prevalence of cryptorchidism and hypospadias are cited as evidence supporting an environmental cause of these diseases (1). For cryptorchidism, the prevalence appears to be highly variable and geographically specific (83), with temporal upward trends noted in some locations but not others (22,84,85). Boisen et al. (83) correlated geographical differences in Denmark and Finland with correspondingly similar trends in testicular cancer and fertility rates. However, in Lithuania, the prevalence of cryptorchidism was higher than expected based on relatively low cancer and high fertility rates (86). Prevalence data for cryptorchidism are hard to interpret, because of the limitations of registry-based data and how they are obtained, changes in clinical practice that emphasize earlier diagnosis and treatment, confounding factors such as birth weight and prematurity, and inaccurate diagnosis related to changes in testicular position (spontaneous descent or secondary "ascent") over time (73). Similarly, data for hypospadias prevalence are

conflicting. Although rates increased in some locations over time, trends were not consistent, and most recent reports identified no changes over time [22,87–89]. Ascertainment bias may also easily exist for this anomaly, particularly for milder forms, as both false-negative and false-positive diagnoses may be made in newborns based on circumcision status.

In summary, animal studies suggest that many eEDCs are capable of altering genital development at high exposure levels. Feminization of the genitalia (shortened anogenital distance with or without hypospadias) is a more common outcome in these studies than prevention of testicular descent (cryptorchidism). By contrast, in human males, cryptorchidism is about 10 times more common than hypospadias, and there have been no consistent trends in the frequency of these common anomalies over time. Nevertheless, susceptibility to the adverse reproductive effects of eEDCs may vary with species and with race/ethnicity differences in human populations. Such differences are compatible with the possibility that eEDCs contribute to the risk of cryptorchidism and hypospadias in human populations.

4.2. Epidemiological Evidence for eEDC Exposure in Males with Cryptorchidism or Hypospadias

Data that directly correlate specific eEDC exposure during pregnancy with fetal reproductive tract anomalies are limited to women treated with DES during pregnancy. Although DES exposure is no longer clinically relevant, study of its effects is useful, as it is a synthetic estrogen agonist that does not bind to maternal plasma proteins and has both anti-androgenic and anti-insl3 effects in animal models. In a large cohort of boys born to women who received high dose DES in a prospective study over 50 years ago, the most common anomalies were epididymal cysts and testicular atrophy, with a risk of cryptorchidism of only 5% *(90)*. Long-term follow-up of this group identified an increased risk of pathological semen but not of failed paternity, other measures of infertility, or of testicular cancer *(91,92)*. Similarly, in a recent review, Storgaard et al. *(93)* found evidence for an association between indices of estrogen exposure and testicular cancer but not cryptorchidism or hypospadias.

Evidence for specific eEDC exposure in males with cryptorchidism or hypospadias is limited. Several studies have failed to identify consistently increased levels of DDT or DDE in serum of mothers bearing offspring with cryptorchidism or hypospadias (Table 4). Longnecker et al. *(94)* interpreted their data as showing a modest association (OR = 1.3, 95% CI = 0.7–2.4) between maternal levels of DDE and cryptorchidism in a large case–control study. In a smaller birth cohort study, maternal serum levels of DDT and DDE during pregnancy did not correlate with the presence of cryptorchidism or hypospadias in male offspring *(95)*. These studies show only small increases in odds ratios for the highest levels of exposure. However, the ability to detect associations may be limited by population heterogeneity and size.

There are very limited data from epidemiologic studies linking exposure to organochlorine compounds with cryptorchidism or hypospadias. Mol et al. *(102)* performed a cohort study of children born in the Faroe Islands, a location of confirmed increased PCB exposure due to dietary intake. Umbilical cord PCB levels were available for the majority of a cohort of 196 boys (75% of those born during a 21-month period in 1986–1987) evaluated for timing of puberty. Nineteen boys (10%) had

a history of cryptorchidism, and half were bilateral. Although this is of high prevalence compared with historical controls, no comparable low exposure group was studied, and PCB levels did not differ between the groups with and without undescended testes. In a small study conducted in Germany (*103*), fat samples were obtained at surgery from boys undergoing orchidopexy for cryptorchidism ($n = 18$) and controls without genital anomalies ($n = 30$). Of a panel of organochlorine compounds measured in these samples including PCBs and DDT and its metabolites, only levels of heptachlorepoxide and hexachlorobenzene, compounds not previously associated with genital anomalies, were significantly elevated in the case group.

The relationship of parental or general community pesticide exposure with hypospadias or cryptorchidism has been explored in several epidemiologic studies (Table 4). Exposures were estimated in these studies based on geographical location (proximity to agricultural activity), parental occupation in agriculture, and/or self-report of exposures. Ascertainment of cases was also varied and based either on newborn exams or medical record documentation of surgical treatment for cryptorchidism. These populations are different, as a proportion of boys undergoing surgery did not have documented cryptorchidism at birth, whereas others identified at birth have spontaneous resolution and do not require surgery (*73*). As such, these studies provide only indirect evidence for exposure to a variety of eEDCs with varying degrees of reproductive toxicity. Even in cases of direct known exposure (e.g., parental occupation as a gardener or farmer), the timing and degree of exposure cannot be retrospectively quantified. Also, as noted by Vidaeff and Sever (*104*), caution must be exercised in interpretation of these studies because of sample size limitations, weak or inconsistent associations, the possibility of recall bias, and/or misclassification of exposure. However, the reported data suggest a possible association between pesticides and the occurrence of one or both genital anomalies. In another series, maternal vegetarianism was significantly correlated with hypospadias in a cohort of 51 affected males (OR = 3.53, 95% CI = 1.56–7.98) and attributed to phytoestrogen exposure, although increased risk related to concomitant pesticide exposure could not be ruled out (*105*). Additional work that directly addresses the association of maternal and/or paternal pesticide exposure with urogenital anomalies by measurement of exposure levels of specific compounds is indicated.

Recent studies indicate that certain phthalate diesters and their metabolites are measurable in breast milk, cord blood, and at higher than expected levels in certain pregnant women (*31*,*106*,*107*), and indirect evidence suggests that certain phthalates may affect Leydig cell function in the perinatal period. In a study of phthalate concentrations in breast milk and hormone levels in 3-month-old male offspring, Main et al. (*106*) identified a significant negative correlation between MBP, the active metabolite of DBP, and free testosterone levels (determined by total testosterone: sex hormone binding globulin ratio). However, phthalate levels did not correlate with the occurrence of cryptorchidism that was present in approximately half of this cohort of boys. Interestingly, levels of MBP were significantly higher in breast milk from Finnish as compared with Danish mothers despite a significantly lower prevalence of cryptorchidism and hypospadias (*86*) in Finland. In another study of phthalate exposure and male genital development, Swan and colleagues (*108*) determined "anogenital index" (anogenital distance/body weight) and testicular position in young boys (mean age 16 months) and corresponding maternal levels of urinary phthalate metabolites at three separate

clinical sites. In this study, the authors found significant inverse relationships between the highest maternal levels of MBP, MBzP, MEP, MiBP, and anogenital index [OR for MBP: 10.2 (95% CI = 2.5–42.2)], although MEP has not been linked to reproductive anomalies in rodent studies. The prevalence of "incomplete testicular descent" ranged from 6 to 20% with decreasing anogenital index, levels that are unusually high and may reflect the high incidence of non-pathological testicular retractility in normal boys of this age *(*[73]*)*. Moreover, in boys, stretched penile length is the standard measure used to assess male genital masculinization; the validity of anogenital index in this regard is well defined in rats but not confirmed in human males. Scrotal anatomy, another endpoint studied that may in part determine anogenital distance, is highly variable and does not necessarily correlate with penile development or testicular descent.

In summary, the strongest epidemiological data that link eEDC exposure to cryptorchidism and/or hypospadias are those suggesting an association between residency in agricultural areas and/or measures of direct parental exposure to non-organochlorine pesticides, without providing insight into specific potentially causative agents. However, these data are not necessarily consistent for both anomalies or congruent with observations made in animal experiments. Further studies will be needed to provide a clearer understanding of the role(s), if any, of specific eEDCs in the etiology of genital anomalies in man.

5. CONCLUSIONS AND CHALLENGES

Throughout this chapter, we have tried to provide insights into the current state of the epidemiologic evidence on the relationship between eEDC and male reproductive and developmental health. The chapter was not meant to be an exhaustive review of the evidence but rather a synthesis of the current knowledge in an ever-changing field of inquiry and discovery. The classes of chemicals included in this chapter represent a number of contemporary and widely used compounds such as pesticides and phthalates. In addition, although PCBs were banned several decades ago, they were also discussed because recently many well-designed epidemiologic studies have been published.

Although there is current scientific, public, and governmental interest in the potential health risks of exposure to eEDCs, the human evidence on associations of eEDCs with altered male reproductive health endpoints remains limited. In addition, the quality and quantity of studies varies widely across exposures and endpoints. For example, although there are hundreds of different pesticides currently in use worldwide, limited human data exists on risks to male reproductive endpoints for only a select few. Also, for some of these chemicals, such as phthalates, the data across studies were not entirely consistent. For instance, one study found associations of semen quality with MBP and MBzP while another large epidemiologic study did not. The limited human data, and in certain instances inconsistent data across studies, highlight the need for further epidemiological research on these classes of eEDCs.

In addition to the classes of eEDCs discussed in this chapter, there are other classes of chemicals that require further study as to their relation with human reproductive and developmental health. These chemicals include, among others, alkylphenols, such as 4-nonylphenol, BPA, and fluorinated organic compounds such as perfluorooctane

octanoate (PFOA) and perfluorooctane sulfonate (PFOS). Alkylphenols are used as surface active agents in cleaning/washing agents, paints, and cosmetics, whereas BPA is used in the manufacture of polycarbonate plastics and epoxy resins. The perfluorinated compounds are used to make fabrics stain resistant/water repellent and in coatings on cookware and other products. Although human exposure to these chemicals has been demonstrated, the epidemiologic evidence on potential health effects remains very limited.

A future challenge in understanding the relationship between eEDC and male reproductive and developmental health includes the changes in exposure levels among populations over time because of the ever-changing patterns of production and use of these compounds. Another challenge is to understand how simultaneous coexposures to these chemicals may affect male reproductive health and development. It is well known that most men are exposed to all of these classes of eEDC simultaneously, as well as to many other chemicals. However, there is limited data on the interactions between chemicals within a class or across classes of chemicals. Chemicals may interact additively or multiplicatively, or antagonistically. An understanding of the potential human health risks of exposure to mixtures of eEDC is important but remains very understudied. Despite these challenges, evolving and innovative technologies designed to improve the assessment of human exposure and male reproductive health endpoints should provide enhanced opportunities for improving our understanding of the relationship between eEDC and health. Innovations include improved biomarkers of exposure and more sophisticated statistical methods that deal with multiple exposures simultaneously.

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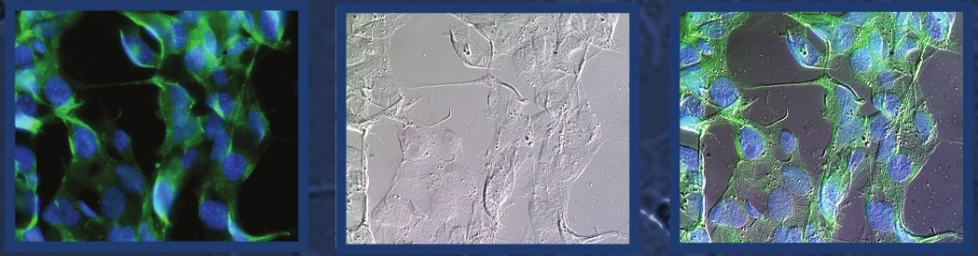

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