

# **Disruption of Androgen Receptor Signaling in Males by Environmental Chemicals**

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## **Abstract**

Androgen-disruptors are environmental chemicals that interfere with the biosynthesis, metabolism or action of endogenous androgens resulting in a deflection from normal male developmental programming and reproductive tract growth and function. Since male sexual differentiation is entirely androgen-dependent, it is highly susceptible to androgen-disruptors. Animal models and epidemiological evidence link exposure to androgen disrupting chemicals with reduced sperm counts, increased infertility, testicular dysgenesis syndrome, and testicular and prostate cancers. Further, there appears to be increased sensitivity to these agents during critical developmental windows when male differentiation is at its peak. A variety of *in vitro* and *in silico* approaches have been used to identify broad classes of androgen disrupting molecules that include organochlorinated pesticides, industrial chemicals, and plasticizers with capacity to ligand the androgen receptor. The vast majority of these synthetic molecules act as anti-androgens. This review will highlight the evidence for androgen disrupting chemicals that act through interference with the androgen receptor, discussing specific compounds for which there is documented *in vivo* evidence for male reproductive tract perturbations.

Keywords: endocrine disrupting chemicals, EDC, androgen-disruptor, androgen receptor, antiandrogen, vinclozolin, DDT, phthalate

## 1. Introduction

Male reproductive health is defined by both the proper development of the reproductive system and maintenance of function throughout adult life, including the capacity to reproduce. While female sexual differentiation, considered the default developmental pathway, is largely independent of estrogens and androgens, male sexual differentiation is driven by androgens produced by the fetal testes and is entirely androgen-dependent [1, 2]. Consequently, it is expected that endocrine-disrupting chemicals (EDCs) that interfere with androgen action will have a greater impact on male developmental programming and reproductive tract maturation.

In contrast to estrogenic modes of action, relatively little is known about how androgenic/antiandrogenic EDCs at environmentally relevant concentrations affect male reproductive tract health. Androgens mediate a wide range of developmental and physiological responses in the male and are crucial for testicular and accessory sex gland development and function, pubertal sexual maturation in multiple organs, maintenance of spermatogenesis and maturation of sperm, male gonadotropin regulation through feedback loops and various male secondary characteristics such as bone mass, musculature, fat distribution and hair patterning [2, 3]. Testosterone and its metabolite 5- $\alpha$ -dihydrotestosterone (DHT), the primary androgenic hormones, mediate their biological effects predominantly through binding of the androgen receptor (AR), which is expressed in many end-organs including the hypothalamus, pituitary, liver, prostate, and testes [3]. There are multiple sites whereby EDCs can interfere with androgen-dependent mechanisms and affect male reproductive tract health and these include androgen synthesis, metabolism and clearance, feedback regulation, AR expression in target organs, and direct AR binding [4-9]. This review will focus on EDCs that ligand the AR and in so doing, behave *in vitro* as AR antagonists and/or, in a few cases, as AR agonists. Further, we will highlight the *in vivo*

evidence that some of these man-made chemicals interfere with biological processes and in so doing, disrupt male reproductive tract health and well-being.

## **2. Androgen Receptor**

The actions of androgens within target cells are transduced by the low abundance intracellular AR, the number 4 member of the NR3C subgroup of a nuclear receptor superfamily that mediates the action of steroid hormones [10]. The human *AR* cDNA was first cloned in 1988 [11, 12] and an AR has since been described in a number of species including, mouse [13], rat [14], rabbit [15] monkey [16] and fish [17, 18]. The single-copy androgen receptor gene is localized on the human X chromosome between q11-q13 [19] and contains 8 exons with a total length of 90 kb. As schematized in Figure 1, the large AR gene encodes a 115-120 kD modular protein with five domains that each harbor an autonomous function that is critical to AR action; an N-terminal or A/B domain (NTD) with transactivation function, the DNA-binding or C domain (DBD), a hinge region or D domain and a ligand-binding or E domain (LBD) [20-22].

The first 30 residues of the AR NTD are highly conserved and critical for interactions with the LBD that provide for agonist-induced stabilization of the receptor [23]. This NTD-LBD interaction between 2 AR molecules is a property unique to AR among the steroid receptor family. The NTD also harbors the transcriptional Activation Function-1 (AF-1) domain which specifies the cell and promoter-specific activity and functions as a site for co-receptor protein interaction. Phosphorylation of the NTD via the actions of multiple intracellular kinases is a well characterized post-translational modification that permits ligand-independent AR activation [23, 24]. The *AR* gene has a unique feature compared to its sex steroid receptor counterparts in that it contains polymorphic repeats of CAG (glutamine) and GGC (glycine) in the NTD, which have been linked to certain chronic diseases [24].

The DBD consists of two zinc-fingers that are encoded by exons 2 and 3, respectively, which recognize and bind to the *cis*-acting enhancer DNA sequences, or hormone response elements (HRE) located within the regulatory regions of target genes. The first zinc-finger composes the P-box (proximal box) conferring specificity sequences on the receptor protein and also forming a “recognition helix” [25]. P-box residues are identical among the AR, progesterone and glucocorticoid receptors, therefore, these receptors bind a common consensus HRE (or GRE). Amino acids of the second zinc-finger form the “D-box” (distal box) and are more specifically involved in spacer sequence and provide an interface for receptor dimerization [25]. The D domain primarily serves to connect the more highly conserved C and E domains of the receptor. Commonly referred to as the “hinge” region, the D domain also harbors a nuclear localization signal that influences cellular compartmentalization of the receptor.

The AR LBD is a highly structured, multifunctional region that primarily serves to bind androgens and also is the primary site for EDC interactions. The LBD of the AR in humans, rats and mice is identical and provides for high affinity binding of two endogenous androgens, testosterone and 5 $\alpha$ -hydroxy-testosterone (DHT), the latter of which binds with much greater affinity [26]. Similar to other steroid receptors, the LBD contains an Activation Function-2 (AF-2) domain located in the C-terminus. While the AF-2 augments ligand-dependent transcriptional activity for most steroid receptors, this function is markedly weaker in the AR where the AF-2 is more involved in interactions with residues in the NTD [23]. Receptor binding to an agonist ligand leads to rearrangement of the LBD such that helix-11 is repositioned and helix-12 swings back to form a “lid” over the binding pocket [27]. This agonist-induced repositioning of helix-12 leads to the formation of a hydrophobic cleft, which serves to recruit co-activators such as p160 to the receptor complex to promote receptor transcriptional activity. In contrast, receptor antagonists are unable to induce a similar repositioning of helix-12, leading to receptor recruitment of co-repressors such as

NcoR and SMRT and a structure incompatible with co-activator recruitment, thus making it less likely to activate transcription [28].

In the “classic” model of steroid receptor action, the AR resides in the nucleus or cytoplasm but is sequestered in a multi-protein inhibitory complex in the absence of hormone. Upon hormone binding, a conformational change occurs in the receptor, transforming it to an “activated” state that is now able to homodimerize, show increased phosphorylation, and bind to HREs within target gene promoters. The ligand/HRE-bound receptor complex interacts with the general transcription apparatus either directly or indirectly via co-regulatory proteins to promote transcription of the target gene [25]. This classic steroid receptor mechanism is dependent on the functions of both AF-1 and AF-2 domains of the receptor, which synergize via the recruitment of co-activator proteins to the DBD, most notably the p160 family members. It is generally believed that the DNA-bound receptor/co-activator complex facilitates disruption of the chromatin and formation of a stable transcription pre-initiation complex. Unique to AR among the sex steroid receptors is that the agonist-liganded AR NTD interacts with co-repressors NCoR and SMRT which function as negative regulators of androgen-stimulated transcriptional activity [28]. Depending on the cell and promoter context, the DNA-bound AR complex may positively or negatively affect expression of the downstream target genes.

### **3. Evidence Linking AR Disruptors with Disorders of Male Health**

The link between environmental chemicals and male infertility has been widely appreciated since 1962 with the publication of Rachel Carson’s book, *Silent Spring*, which highlighted the effects of dichlorodiphenyltrichloroethanes (DDTs) on infertility in birds and other wildlife. While human studies examining altered male reproduction in relation to environmental chemicals were initially limited, evidence has emerged over the years that suggests a link between hormonally active toxicants and developmental reproductive

abnormalities [29]. Studies have raised the possibility that EDCs may be contributing to a decline in the human sperm count that has been observed over the last 50-60 years [30-32]. A review of 61 international studies involving 14,947 men between 1938 and 1992 showed that the average sperm count had dropped from 113 million/ml in 1940 to 66 million/ml in 1990, in addition to alterations in sperm morphology and motility [33]. Many epidemiological studies suggest a link between non-persistent (or ‘contemporary-use’) pesticide exposure and altered semen quality [34] suggesting that EDCs may be the proximate cause. Two recent studies based on occupational reports involving simultaneous exposure to several pesticides found associations between pesticide exposures representative of that seen in the general population and reduced semen quality [35, 36].

In addition to altered semen quality, other male reproductive tract anomalies potentially attributable to EDCs have emerged with increased frequency over the past few decades that together have been described as testicular dysgenesis syndrome [37]. These disorders in the human population, which include increased incidences in cryptorchidism, hypospadias, oligospermia, and testicular germ cell cancer, have been linked in some studies to prenatal endocrine-disruptor exposure [37-43]. It is interesting to note that the so-called testicular dysgenesis syndrome has geographical specificity which emphasizes the likelihood that environmental factors contribute to these reproductive tract abnormalities [44]. Although not all cases of these disorders are a result of EDC exposures and have separate etiologies, a unifying hypothesis that links these disorders provides an intriguing and compelling argument that requires further investigation. Other male reproductive abnormalities that have been associated with EDCs that have AR disruptor activity in both human epidemiology studies as well as in animal models included delayed puberty [45] and reduced anogenital distance in newborn boys [46].

Another area of male reproductive health potentially linked to EDC exposures is cancers of the reproductive tract, specifically testicular and prostate cancers. Testicular

cancer rates have increased worldwide over the past 35 years with the greatest increase observed in certain European populations. Although direct evidence for increased testicular cancer due to EDC exposures is very limited, there are several reports [47, 48] that suggest a link. *In utero* diethylstilbestrol exposure has been associated with an increased risk of testicular cancers [49] while maternal levels of chlorinated chemicals suggests a link for these compounds with mixed estrogenic and antiandrogenic activity to testicular cancer rates in sons [50]. Further, a rabbit model for testicular cancer identified exposure to di-*n*-butylphthalates with antiandrogenic action to testicular carcinoma *in situ* [51]. There is compelling data for increased prostate cancer risk and exposure of farmers to pesticides, some which are inhibitors of p450 enzymes involved in steroid metabolism [42, 50, 52]. Epidemiologic studies of occupational exposure to PCBs revealed a strong exposure-response relationship for prostate cancer risk [53] and prostate cancer mortality [54]. While estrogenic activity of these compounds is a suspected mode of action, there is also evidence that some PCBs may behave as antiandrogens.

While there are many sites of action for chemicals to interfere with androgen signaling, available evidence primarily classifies these compounds into two broad categories; (i) interference with androgen biosynthesis or metabolism to indirectly modulate androgen function (nonreceptor-mediated disruptors) and (ii) interaction with the androgen receptor to interfere with the ligand-dependent transcriptional function (receptor-mediated disruptors). Furthermore, it has been shown that some pesticides can act by reducing androgen receptor expression [4-6]. For the purposes of this review, we here look at the exposure to endocrine-disrupting pollutants with identified antiandrogenic toxicity, mostly through binding of the androgen receptor to alter proper folding of its ligand-binding domain (LBD), blocking recruitment of co-activators and preventing transcriptional initiation. Androgen-disruptors acting via this mechanism include vinclozolin, DDT, procymidone, linuron, lindane, dieldrin/aldrin, methoxychlor, nonylphenol, and bisphenol-A [7-9]; Table 1). These

chemicals will be discussed individually in the following section based upon their chemical classifications.

#### 4. Classification of AR Disrupting Chemicals

Androgen receptor-mediated disruptors can be classified into agonists and antagonists. An agonist binds to androgen receptor and triggers a response mimicking the action of a naturally occurring androgen. In contrast, an antagonist acts opposite to an agonist and blocks androgen receptor transactivation. Thus far, there are limited studies on screening of androgen receptor binding activity for a large number of chemicals [55-62], which include studies based solely on their chemical structure [63, 64]. Among the systematic investigation of compounds, a pilot study by Araki and colleagues [55] is the first report of industrial or environmental chemicals with AR agonist activity. However, not until a few years ago was 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH; Figure 2) identified as the first potent environmental activator of the human AR [65] and the only AR agonist for which *in vivo* study has been performed [66].

##### 4.1. Agonists

###### 4.1.1. Tetrabromoethylcyclohexanes (TBECHs)

TBECH, a brominated flame retardant used in a variety of products as insulation, stuffing in furnishings, is present in both sediments and organisms along with its potent activation of AR at nanomolar concentrations caused TBECH to be ranked as one of the 10% most hazardous compounds to ecosystems [67]. TBECH can exist in four diastereoisomeric forms:  $\alpha$  and  $\beta$ , found in the commercial flame retardant marketed as Saytex BCL 462 by Albemarle Corp.; and  $\gamma$  and  $\delta$ , converted from  $\alpha$  and  $\beta$  at temperatures  $> 120^{\circ}\text{C}$  [68].

*In silico* analysis of interaction energies and *in vitro* binding assays showed that TBECH diastereomers  $\gamma$  and  $\delta$  are more potent activators of the human AR than  $\alpha$  or  $\beta$  [69].

TBECH- $\gamma\delta$  (50:50) binds to the AR with 22% of DHT's binding affinity and all diastereomers induced expression of the downstream target prostate-specific antigen (PSA) *in vitro* [69]. Nyholm and colleagues [66] showed that TBECH can be maternally transferred in zebrafish. Future studies are needed to determine the androgenic effects of TBECH *in vivo*.

## 4.2. Antagonists

Environmental and industrial chemicals with antiandrogenic action cover wide range of chemical structures including flutamide derivatives, diphenylmethanes, phthalates, organochlorines, and alkylphenols (Figure 2).

### 4.2.1. Diphenylmethanes

#### *Dichlorodiphenyltrichloroethanes (DDTs) and Congeners*

Commercial DDT contains several isomers of which *p,p'*-1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane (*p,p'*-DDT) and its persistent metabolic derivative *p,p'*-1,1-Dichloro-2,2-bis(*p*-chloroethyl)ethylene (*p,p'*-DDE) are the major components. DDT was widely used as a pesticide in the United States until its ban in 1972 after it was found to have adverse effects on male reproductive tract development in wildlife [9, 70]. The Second National Health and Nutrition Examination Survey conducted between 1976 and 1980 revealed a prevalent human exposure in 99.5% of the U.S. population with measurable serum *p,p'*-DDE levels [71]. Although human exposure has declined significantly since that time, some populations still bear significant levels of *p,p'*-DDE [72, 73] due to its considerably high environmental half-life, bioaccumulation and the continued use of DDT against malaria in some developing countries.

Regarding its properties as an AR disruptor, DDT isomers *p,p'*-DDT, *o,p'*-1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane (*o,p'*-DDT) [74], and metabolite *p,p'*-DDE [57] were shown to reduce binding of DHT to AR *in vivo* while *p,p'*-DDE inhibited DHT-induced

transcriptional activation *in vitro* [9]. In addition to AR antagonistic effects of DDT, *p,p'*-DDE at high concentrations has been shown to function as an inhibitor of 5 $\alpha$ -reductase, responsible for converting testosterone to DHT [75] thus it is likely that these compounds interfere with androgen signaling at multiple sites of action.

Although human serum concentrations of DDT and *p,p'*-DDE were only weakly associated to cryptorchidism or hypospadias in offspring [76-79], fetal and neonatal exposure in male produced demasculinizing effects with a high incidence of epididymal and testicular lesions [9, 80], and reduced prostate growth and inflammation [81].

#### *Methoxychlor*

The insecticide methoxychlor is structurally related to DDT. Compared to DDT, however, it has low environmental persistence and therefore was used to replace DDT as a pesticide after the later was banned in the United States. Beyond its considered estrogenic activity, methoxychlor also shows affinity to the AR at comparable or even higher levels than DDTs [57]. While methoxychlor exposure of neonatal rats did not affect male puberty, reproductive organ weights or functions in adulthood [82, 83], exposure throughout gestation, weaning, and lactation resulted in multiple effects including inhibition of somatic growth and accessory sex gland weight, elevated pituitary and serum prolactin levels, delayed puberty, suppression of Leydig cell function, reduced sperm counts [84, 85] and decreased DNA content of the accessory sex glands in the male offspring [86]. Rats fed 2000 ppm methoxychlor for 90 days exhibited decreased prostate size and cell content [87]. Together, these biological endpoints indicate robust antiandrogenic activity of this environmental contaminant with regards to male reproductive health.

#### *Bisphenol A (BPA)*

BPA is a synthetic polymer used in the production of polycarbonate plastics and epoxy resins and significant levels have been found in the urine of 93% of the US population in a recent screen by the CDC [88]. While its mode of action is believed to be primarily as an

estrogen receptor agonist, studies have also shown AR binding [57] and antagonistic activity for BPA [89]. Thus negative effects of BPA on male reproductive health need to be evaluated in the context of its antiandrogenic capacity in addition to its estrogenic actions. BPA exposures have been linked to reduced sperm counts in a rodent model [90] and a human epidemiology study [91]. Recent assessment of occupational exposure to BPA has linked erectile dysfunction in men with high urinary BPA levels [63].

The effects of BPA with regards to carcinogenic potential, including the prostate gland, have recently been reviewed by an expert panel [92]. In short, there is evidence from rodent models and human prostate cell lines that BPA can influence carcinogenesis, modulate prostate cancer cell proliferation, and for some tumors, stimulate progression [93, Prins, 2008 #3196, 94]. Of particular interest with regards to AR action are the studies by Knudsen and colleagues who examined the influence of BPA on human prostate cancer cells that contained an AR point mutation (AR-T877A) frequently found in advanced prostate cancers of patients who relapse after androgen deprivation therapy [95]. They first observed that 1 nM BPA activates AR-T877A in transcriptional assays and leads to unscheduled cell cycle progression and cellular proliferation *in vitro* in the absence of androgen. Since BPA had no impact on wild-type AR, these data indicate that this *gain-of-function* AR mutant attained the ability to utilize BPA as agonist. Subsequent *in vivo* analyses of the impact of BPA on human prostate tumor growth and recurrence were performed using a mouse xenograft of human cells containing the AR-T877A mutation [94]. At low doses that fall within the reported ranges of human exposure, prostate tumor size increased in response to BPA administration. Further, mice in the BPA cohort demonstrated an earlier rise in PSA which is indicative of biochemical failure. These outcomes underscore the need for further study of the effects of BPA on prostate tumor progression and therapeutic efficacy.

#### 4.2.2. Flutamides

##### *Vinclozolin*

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-oxazolidine-2,4-dione] is a systemic dicarboximide fungicide extensively used to control diseases caused by *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Monilinia spp.* on fruits, vegetables, ornamental plants and vines. It is a well known contaminant of the human diet with endocrine disrupting potential as an AR antagonist through its primary metabolites 2-[[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenic acid and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide, [96]. Vinclozolin has been shown to inhibit AR transactivation and androgen-dependent gene expression [96-98]. Defects in prostate development and ectopic location of the testes are equivalent to those observed in rats treated with the antiandrogen flutamide, suggesting a similar mechanism of action.

*In vivo* administration of vinclozolin at different doses, routes, and periods (gestation, lactation, puberty, adulthood) dictates the effects on the male reproductive tract. Chronic prostatitis induced by transient *in utero* exposure during late gestation (days 14-19) in rats was not evident until puberty or thereafter and are reported to be reversible by pubertal androgen treatments [99]. Peripubertal oral administration of vinclozolin delayed puberty and altered sex accessory gland and epididymal growth in male rats [100]. In contrast, exposure of rats to vinclozolin during midgestation, time of sex determination, promoted multiple adult-onset phenotypes including penile malformation, decreased sperm production and motility, increased spermatogenic cell apoptosis, altered sperm maturation proteins, hypospadias, cleft phallus, suprainguinal ectopic testes, vaginal pouch, epididymal and testicular granulomas, atrophic accessory sex glands, and kidney disease with tumor development [80, 99, 101-106]. In studies performed by Skinner and colleagues, the developmental vinclozolin exposure effects were found to be heritable through multiple generations with continued defects observed in the F3 generation and beyond [107]. An

epigenetic basis for the transgenerational disease phenotype of vinclozolin has been established that involves perturbations in DNA methylation patterns on the male germ cell [108]. It is important to note that timing of the exposure is critical for the epigenetic and transgenerational effects on vinclozolin and is related to the establishment of DNA methylation patterns during sex determination in developing male germ cells on fetal days 8-14.

### *Linuron*

Linuron is currently marketed as a selective phenyl urea herbicide for pre- and/or post-emergence control of weeds in crops. The structurally related diuron is used to keep weeds from track systems and sporting grounds [109]. Both compounds are degraded in the environment to 3,4-dichloroanilide and further metabolized into 3,4-dichloroacetanilide [109].

Linuron displays weak affinity to AR [57, 110-112]. In a 2-year feeding trial, linuron increased the incidence of testicular tumors in rats [111]. In short-term *in vivo* dosing, linuron treatment reduced testosterone- and DHT-dependent tissue weights [80] and altered the expression of androgen-regulated rat ventral prostate genes [113]. *In utero* exposure to linuron (day 14–18) induced developmental alterations of the testes and epididymides in the male rat offspring [113].

### 4.2.3. Organochlorines

#### *Hexachlorocyclohexanes*

Commercial lindane is a hexachlorocyclohexane (HCH) and consists of several isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ). The  $\gamma$ -HCH form, which is used as an insecticide, tends to accumulate in body fat of mammalian species and is the most acutely toxic [114, 115]. It has been shown that lindane binds to AR in rat prostate [57, 116], inhibits DHT binding [74], and causes

biochemical and histological changes in the rat testis [117]. The  $\beta$ -HCH isomer is a byproduct in the manufacture of lindane and accounts for 90% of the total HCH found in human milk [115]. Male rats fed with  $\beta$ -HCH throughout lactation and weaning developed reproductive toxicity characterized by reduced size of seminiferous tubules and decreased number of interstitial cells along with spermatogenic arrest [Van Velsen, 1986 #3495; [118].

### *Procymidone*

Procymidone is a dicarboximide fungicide structurally related to the well-characterized fungicide vinclozolin. It has low potential for bioaccumulation in the soil and moderate mobility. *In vitro*, procymidone binds AR [57, 112, 119], inhibits DHT binding in transfected COS (monkey kidney) cells, and inhibits DHT-induced transcriptional activation in CV-1 cells [8].

Procymidone causes reproductive malformations in the fetal male at dosage levels that have little effects on the reproductive tract of the adult male rat [8, 80, 119]. Maternal procymidone exposure during gestation and early lactation caused shortened anogenital distance, permanent nipples, reduced weight of several androgen-dependent tissues (levator ani and bulbocavernosus muscles, prostate, seminal vesicles, Cowper's gland and glans penis), and reproductive tract malformations (hypospadias, cleft phallus, vaginal pouch, hydronephrosis, occasional hydroureter, epididymal granulomas, and ectopic, undescended testes). In addition, perinatal procymidone treatment had a marked effect on the histology of the lateral and ventral prostatic and seminal vesicular tissues displaying increased incidence of inflammation, similar to that produced by perinatal exposure to vinclozolin [8, 80].

### *Aldrin and Dieldrin*

Aldrin and its major metabolite, dieldrin, were used as insecticides until the early 1970s, when they were widely restricted or banned. Aldrin is rarely found in food but dieldrin

accumulates in the mammalian organism causing background levels in the environment [115]. *In vitro*, aldrin binds AR [57] and dieldrin reduces binding of DHT to AR [74]. In most of the reproduction studies (over one to six generations) carried out with aldrin or dieldrin on mice and rats, the major effect was an increased mortality rate in pups not yet weaned. Reproductive performance was only affected at doses causing maternal intoxication. Single doses of aldrin and dieldrin, equal to about half the LD<sub>50</sub>, caused severe fetotoxicity and an increased incidence of teratogenic abnormalities in the mouse and hamster [120]. Mating studies of dieldrin-exposed rats suggest male-dependent disturbances in fertility [121].

#### 4.2.4. Phthalates

##### *Butylbenzylphthalate (BBP)*

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. They 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 [122]. They are also used 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 release of some pharmaceuticals [122].

BBP was shown to binds AR *in vitro* [57]. *In utero* or perinatal BBP exposure of rats produced a diverse profile of reproductive malformations in the male offspring mainly characterized by reduced weight of the testis, epididymis, ventral prostate and glans penis, reduced anogenital distance, female-like areolas/nipples, and decreased daily sperm production [80]. A large study on male partners of subfertile couples from an infertility clinic in Massachusetts [123, 124] found a dose-response relationship between monobenzyl phthalate (MBzP, the primary hydrolytic metabolite of BBP) and sperm concentrations that

fell below the WHO reference value. In contrast to the U.S. study, a Swedish study found no relationship between MBzP levels and any semen parameter [125]. The Swedish study population consisted of young men (median age, 18 years-old; range, 18–21) from the general population, whereas in the U.S. study the median age of the men from an infertility clinic was 35.5 years-old (ranged from 22 to 54). It is unclear whether middle-aged men, compared with young men, are more susceptible to reproductive toxicants.

#### *4.2.5. Alkylphenols*

Alkylphenol ethoxylate (APE) are surfactants widely used as industrial (such as textile and paper industry) and laboratory detergents, antioxidant, plastic stabilizers, as well as carriers in agricultural pesticides. In sewage treatment plant effluents, APEs are degraded to more stable, persistent, and hydrophobic alkylphenols such as 4-*n*-nonylphenol (nNP) and 4-*n*-octylphenol (nOP). Although alkyl phenols are mainly known for their estrogenic properties, nNP and nOP have been found to be weakly antiandrogenic in recombinant yeast reporter gene assays [8, 59, 89, 126]. Neonatal exposure to nOP negatively affected pubertal spermatogenesis by significant advanced lumen formation and decreased apoptotic rate of germ cells [127], and reduced plasma testosterone [128] in male rat offspring. Male rats exposed during gestation or during the first 21 days of postnatal life caused reduction in testicular size, ventral prostate weight, and daily sperm production [129].

## **5. Summary**

This review has synthesized the current evidence for EDCs acting as disruptors of androgen signaling in the male reproductive tract. Although the list is not comprehensive, it is clear that sufficient data has accrued to indicate that environmental contaminants are capable of deleterious effects on male reproductive tract health through their abilities to act as AR antagonists, or in a few cases, as AR agonists. While mechanistic data mostly arises from in

vitro assays and in vivo animal models, there are an increasing number of human studies and epidemiology reports that document clear negative impacts on the human male. While further research at all levels is required to provide detailed understandings of mechanisms and impacts, it is important to take caution at this stage with the continued use of environmental compounds that disrupt male reproductive health. Importantly, continued studies are required to monitor the use of the known compounds as well as to screen all chemicals for potential AR disrupting activity so that caution may be applied with their continued use.

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## Figure Legends

**Figure 1.** Domain structure of the androgen receptor. The androgen receptor is composed of a N-terminal domain (NTD) or A/B domain, with transactivation function mediated through the AF-1 region, a DNA-binding (DBD) or C domain, harboring two zinc fingers that recognize AREs in regulated genes, a hinge region or D domain, and a ligand-binding (LBD) or E domain that contains the steroid binding pocket and helices 11 and 12 as well as the activation function-2 region (AF-2).

**Figure 2.** Two-dimensional molecular structures of selected androgen disruptors from each class. DHT, dihydrotestosterone; TBECH, tetrabromoethylcyclohexane; *p,p'*-DDE, *p,p'*-1,1-Dichloro-2,2-bis(*p*-chloroethyl)ethylene; nOP, 4-*n*-octylphenol

**Table 1.** Endocrine-disrupting pollutants with antiandrogenic toxicity through binding of the AR.

Androgen-disruptor	Effects and associated mechanism	References
<i>Diphenylmethanes</i>		
DDT	Decreased fertility, cryptorchidism; inhibition of DHT binding to AR, perturbed Ca <sup>++</sup> mobilization, phosphorylation of c-ERB2/c-met; inhibition of p450 <sub>scc</sub>	[9, 74, 130-134]
DDE	Cryptorchidism; inhibition of DHT binding to AR, MAPK pathway and PI3K activation, interacts with GPR30	[9, 74, 133-136]
Methoxychlor	Inhibition of somatic growth, reduced accessory sex gland weight, elevation of serum prolactin; suppression of Leydig cell function; inhibition of spermatogenesis, decreased seminal vesicles and epididymal weight, pubertal delay; induction of CYP28, CYP23A and CAR, alterations in germline DNA methylation, ER activation, antiandrogen and transgenerational effects	[82-85, 87]
Bisphenol A (BPA)	Aberrant development of prostate and urethra, increased anogenital distance, altered periductal stroma, increases susceptibility to prostate hormonal carcinogenesis, inhibition of DHT binding to AR, ligands mutated ARs	[93, 95, 134, 137-139]
<i>Flutamides</i>		
Vinclozolin	Hypospadias, undescended testes, delayed puberty, prostate disease among subsequent generations; inhibition of DHT binding to AR, alters germline DNA methylation patterns	[100, 134, 140-143]
Linuron	Disruption of reproductive tract development; reduction of epididymal and accessory sex gland weight; increased serum estradiol and luteinizing hormone	[113]
<i>Organochlorines</i>		
Lindan ( $\gamma$ -HCH)	Alterations in testes histology; inhibition of DHT binding to AR	[74, 117]
Procymidone	Fetal rat: shortened anogenital distance, permanent nipples, hypospadias, ectopic undescended testes, reduced weight and altered histology of prostate (and several other androgen-dependent tissues)	[8]
Dieldrin/aldrin	Reproductive performance affected at doses causing maternal intoxication; inhibition of DHT binding to AR	[74]
<i>Phthalates</i>		
Butylbenzylphthalate	Reduced anogenital distance and weights of testes, epididymis, ventral prostate, and glans penis, female-like areolas/nipples, hypospadias, cryptorchidism, oligospermia, infertility; inhibition of DHT binding to AR	[80, 134, 144, 145]
<i>Others</i>		
Nonylphenol	MAPK pathway activation, PKC/cAMP modulation, reduced CYP1A1 expression; Reduction of 5 $\alpha$ -reductase and 3 $\alpha$ -hydroxysteroid-dehydrogenase activities; inhibition of DHT binding to AR	[7, 8]