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Untangling the association between environmental endocrine disruptive chemicals and the etiology of male genitourinary cancers

Tiffani J Houston[1,6], **Rita Ghosh**[2,3,4,5,*]

[1]Department of Cell Systems and Anatomy, The University of Texas Health at San Antonio, San Antonio, Texas, USA

[2]Department of Urology, The University of Texas Health at San Antonio, San Antonio, Texas, USA

[3]Department of Molecular Medicine, The University of Texas Health at San Antonio, San Antonio, Texas, USA

[4]Department of Pharmacology, The University of Texas Health at San Antonio, San Antonio, Texas, USA

[5]Department of UTHSA Cancer Center, The University of Texas Health at San Antonio, San Antonio, Texas, USA

[6]Department of South Texas Medical Scientist Training Program, The University of Texas Health at San Antonio, San Antonio, Texas, USA

Abstract

Endocrine disrupting chemicals disrupt normal physiological function of endogenous hormones, their receptors, and signaling pathways of the endocrine system. Most endocrine disrupting chemicals exhibit estrogen/androgen agonistic and antagonistic activities that impinge upon hormone receptors and related pathways. Humans are exposed to endocrine disrupting chemicals through food, water and air, affecting the synthesis, release, transport, metabolism, binding, function and elimination of naturally occurring hormones. The urogenital organs function as sources of steroid hormones, are targeted end organs, and participate within systemic feedback loops within the endocrine system. The effects of endocrine disruptors can ultimately alter cellular homeostasis leading to a broad range of health effects, including malignancy. Human cancer is characterized by uncontrolled cell proliferation, mechanisms opposing cell-death, development of immortality, induction of angiogenesis, and promotion of invasion/metastasis. While hormonal malignancies of the male genitourinary organs are the second most common types of cancer, the molecular effects of endocrine disrupting chemicals in hormone-driven cancers has yet to be fully

[*]**Correspondence:** Rita Ghosh, PhD, Department of Urology, Mail code 8252, 8403 Floyd Curl Drive, San Antonio, TX 78229-3900, Phone: (210) 562-4117, Fax: (210) 562-4133, ghoshr@uthscsa.edu.

TJH did the literature searches and wrote the manuscript

RG developed the theme, edited the manuscript and supervised the work

explored. In this commentary, we examine the molecular evidence for the involvement of endocrine disrupting chemicals in the genesis and progression of hormone-driven cancers in the prostate, testes, and bladder. We also report on challenges that have to be overcome to drive our understanding of these chemicals and explore the potential avenues of discovery that could ultimately allow the development of tools to prevent cancer in populations where exposure is inevitable.

1. The pervasive nature of endocrine disrupting chemicals

Endocrine disrupting chemicals (EDCs) are identified as compounds that modify hormonal and homeostatic systems [1]. The United States Environmental Protection Agency (EPA) describes EDCs as exogenous agents that interfere with endogenous hormones, thus altering homeostasis, deregulating developmental processes [2], and disrupting numerous other mechanisms that merge upon the endocrine and reproductive systems. The molecules classified as EDCs are heterogeneous in nature and include synthetic compounds such as chemicals within industrial solvents/lubricants and their by-products, polychlorinated biphenyls and other plasticizers, pesticides, and pharmaceuticals. Natural molecules with endocrine disrupting properties include heavy metals, such as, arsenic, cadmium, and lead. Although phytoestrogens, such as those found in soybeans and its products, causing alterations in hormonal processes have been reported, their endocrine activity is still controversial. In a 2009 comprehensive review published by The Endocrine Society [3], experts outlined major sources of EDCs as ramifications of industrialization. Developed societies are characterized by a modern landscape, but with a wide range of industrial chemicals that have contaminated soil and groundwater. This has led to persistent biomass accumulation in animals and humans. EDC containing compounds have wide-spread use and many are developed to have long half-lives for industrial purposes. This is evidenced by persistently high levels of substances in the environment that were banned decades ago or have such broad use that they are now detected in previously uncontaminated environments. Most often EDCs exhibit either estrogen/androgen receptor agonist or antagonist activity. EDCs act via a variety of nuclear, non-nuclear, or neuronal receptors as well as enzymatic pathways involved in steroid biosynthesis and/or metabolism (Table 1). They also alter the synthesis, release, transport, metabolism, binding, action and elimination of naturally occurring hormones [2]. EDCs have profound systemic consequences due to the influence of the endocrine system on every bodily organ. While recognizing that large numbers of endocrine active substances in our environment are from endogenous sources excreted from various species, this commentary focuses on EDCs which are defined as agents from exogenous sources.

Epidemiological evidence continues to reveal the link between the exposure to EDCs and carcinogenesis. The datasets are abundant and are accompanied by biostatistical studies. The Wingspread Conference of 1991 was the first scientific meeting to acknowledge the deleterious effects of hormone-like chemicals on humans and wildlife [2]. Since the Wingspread Conference, studies in basic science and epidemiology have reinforced the role of EDCs in overall pathophysiology. Research efforts increased following the 2009 Endocrine Society Statement on EDCs [3]. Understandably, developed countries have higher

exposures to EDCs due to industrialization. These same countries have higher incidences of malignancy, more specifically, hormone related malignancies [4].

2.A. Wide-scale biological consequences of EDC exposure

Exposures have been linked to chronic diseases such as obesity, diabetes and metabolic syndrome, reproductive anomalies, developmental aberrations, and neurological defects [1]. Among the health effects, EDC exposure has also been attributed to an increased risk of cancer, particularly those derived from hormone-dependent tissues [1]. Distinct features characterize cancer development including uncontrolled cell proliferation, inhibition of cell-death, induction of angiogenesis, changes in metabolism and invasion/metastasis [5]. Studies investigating the role of EDCs in cancer development do not explore effects that drive aberrations in all these cancer-defining, biological endpoints.

Organs within the urogenital (GU) system are key players within the endocrine system as sources of steroid hormones, targeted end organs, as well as participants within systemic feedback loops. Combined, malignancies of the prostate, bladder, and testes are the second most common causes of all cancers. Molecular studies considering the genetic cause of these tumors are abundant and have directed the development of various preventative and therapeutic modalities. However, there should be increased consideration of the role of environmental factors, such as EDCs, in the development of GU malignancies. In this commentary, we aim to summarize the molecular evidence of EDCs in the etiology of male hormonal cancers to identify avenues for prevention and therapeutics.

2.B. Why prostate, testicular, and bladder cancer?

The macro- and micro- anatomy of the male urologic organs are well-defined, which allows investigation of molecular aberrations during carcinogenesis. Prostate cancer (PCa) ranks as the number one most common non-skin cancer and number two most common cause of cancer death in men. Bladder cancer (BlCa) ranks as the fourth most common cancer and is characterized as a malignancy of men above 50 years old. Bladder tumors have a high recurrence rate and require long-term surveillance, marking it one of the most expensive cancer treatments per capita, adding an estimated 3-billion-dollar annual cost to the health care system [6]. Testicular cancer (TCa) is not as common and has a 95% cure rate if caught in early stages (degree of invasion/metastasis); however, the incidence rate of TCa has been increasing in the United States for many decades and most often afflicts young and middle-aged men. While strides in cancer therapeutics have lowered the overall cancer related deaths by twenty percent in the last 25 years, cancer remains the second leading cause of death in the United States. A better understanding of the molecular mechanisms of carcinogenesis can lead to modifying exposure to environmental risk factors and cancer prevention. Below we review the data of the molecular effects of common EDCs on the prostate, testes, and the bladder as reported in the literature (Table 2 and 3). Assessment of the literature is meant to underscore the importance of EDCs in the etiology of male urological malignancies and identify knowledge gaps in the field.

3A. Bisphenol A

Bisphenol A (BPA) is perhaps the most widely studied endocrine disruptor in carcinogenesis. BPA is used in the production of polycarbonate plastics, epoxy resins, dental sealants and composites, and thermal receipt paper [7]. BPA in food and beverages accounts for the majority of daily human exposure [8] and can migrate into food and beverages from containers with internal epoxy resin coatings as well as from consumer products made of polycarbonate plastic such as baby bottles, tableware, food containers, and water bottles. Incomplete polymerization of BPA leads to leaching of the chemical and subsequent human exposure. It appears that the quantity of BPA that migrates from polycarbonate containers into liquid is based on the temperature of the liquid and is less dependent on the age of the container [8]. As discussed in the National Toxicology brief on BPA, there are differences between rodent models and humans with regards to BPA elimination [8]. Considering oral intake as the most common exposure, BPA undergoes first pass metabolism with glucuronidation to form BPA glucuronide (BPAG) in the liver and is circulated to bodily tissues and the GI tract through bile. The metabolism of BPA may be significantly affected by an individual's genotype [9] and the biological effects of BPAG within organ systems is pending further investigation. Once reaching the kidney, elimination is mostly through urine in humans. In rodents, BPAG is either excreted through the feces or is hydrolyzed and reabsorbed into the GI tract [reviewed in 8]. Interestingly, the GI tract also produces BPAG and upon absorption, distributes to the body (for urinary excretion) or secretes back into the GI tract for fecal excretion or continuation of the cycle [9]. According to the National Health and Nutrition Examination Survey of data from 2003–2004, the CDC estimates 93 percent of Americans age six and older have detectable BPA in the urine [10].

There is controversy over whether or not BPA exerts negative effects on human physiology. While the EPA established a TDI dose of <50μg/kg/day and has maintained that standard [11], the European Food Safety Agency (EFSA) down-regulated the tolerable daily intake (TDI) from 50 to 4 μg/kg/day in 2015. Currently, the FDA has deemed current food levels as safe for consumption based on ongoing safety review of scientific evidence to date. Some view this conclusion as being based on the lack of replication in blinded studies of its deleterious effects conducted in industry-funded laboratories [12]. Of note, in their decision making, regulatory agencies do not consider studies that fail to meet specific criteria, which has the potential to omit findings from hundreds of other peer-reviewed contributions. One criterion is that laboratories be bound by Good Laboratory Practices, which has the potential to exclude data from many academic and government funded laboratories [12]. Another criterion is adherence to agency guidelines in experimental design, assessment, and endpoints. This restriction may limit the ability to investigate other markers of disease pathophysiology as well as appreciate the non-monotonic dose-response curves that have been observed in BPA toxicology [13, 14]. Final conclusions that intergrate the results from the Consortium Linking Academic and Regulatory Insights on BPA Toxicity" (CLARITY-BPA) core study research report and grantee studies data are scheduled to be released in late 2019 (https://ntp.niehs.nih.gov/go/bpa).

With regards to specific EDC effects, although BPA had long been accepted to function as an environmentally active estrogen, recent studies have shown it to bind to and activate not

only nuclear estrogen receptors (ER) α and β, but also the membrane bound, G-coupled protein receptor (GPER), thyroid hormone receptor, androgen receptor (AR), and estrogen-related receptor (ERR)γ [15]. While previous studies have characterized BPA as a weak estrogen agonist, it is now reported that it may be equipotent with estradiol due to rapid induction of non-genomic responses from membrane surface receptors [16, 17].

3A. 1. Bisphenol A: Prostate

Most molecular evidence on BPA and the prostate are reported effects following prenatal exposure. In one of the earliest studies, prenatal BPA exposure at 25μg/kg/day in rats altered phenotype of prostate periductal stromal cells, prostatic functional activity, and tissue organization [18]. A groundbreaking study conducted by Ho et al. shows that neonatal Sprague-Dawley rats exposed to BPA at 10μg/kg/day actually develop high-grade PIN lesions [19]. Epigenetic analysis of the tissues shows that prostates exposed to BPA have aberrant methylation of CpG islands in phosphodiesterase type 4 variant 4 (PDE4D4), an enzyme responsible for cyclic AMP breakdown (cAMP). Dysregulation of cAMP second messenger signaling has implications for unchecked cellular proliferation. Furthermore, prolonged neonatal BPA exposure-associated hypomethylation at this site resulted in increased PDE4D4 expression and promotion of prostatic disease with age [19]. Of note, prior to extrapolating pharmacokinetic evidence from rodent models to study the cancer risks of BPA exposures in humans, the study design must account for the differing metabolism, clearance, and excretion mechanisms between humans and rodents [20]. This distinction is important because conclusions that do not account for differences in BPA metabolism and excretion in data extrapolation can potentially lead to misleading data interpretation. This underscores the need for appropriate models to study environmental exposures and is a major limitation in EDC research. In further advances, a unique human cell culture model used prostate epithelial stem-like cells combined with rat mesenchyme grown as renal grafts to assess *in vivo* early-life carcinogenicity of BPA exposure [21]. Utilizing doses previously described in early-life rodent models that had detrimental prostatic effects, researchers found significant increase of high-grade PIN and adenocarcinoma in grafts exposed *in vivo.* Continuous *in vitro* exposure further increased high-grade PIN incidence [21]. Examination of epigenetic effects following BPA exposure using neonatal rodent prostate tissues reveal DNA methylome changes in genes associated with shorter recurrence-free survival in humans [22]. In fact, developmental BPA exposures that induce PIN and carcinoma are paralleled by nonmonotonic and dose-specific DNA hypomethylation of genes that pose carcinogenic risk. Interestingly, the greatest degree of hypomethylation is found at the lowest BPA doses, suggesting more biological relevance to everyday human exposure [23]. In the first study assessing BPA in the adult male rat, aromatase mRNA levels increase, while 5α-reductase levels have differential expression of isozymes based on testosterone level. Rats were exposed to BPA at 25μg/kg/day and the TDI dose of 50μg/kg/day [24]. 5α-reductase-R3 mRNA increases with decreased levels of testosterone, which is similar to the observations seen in the progression of PCa [25]. A recent study using a novel gerbil *(Meriones unguiculatus)* animal model to assess changes in prostate morphology and histopathology found long-term exposure to environmentally relevant doses of BPA (50μg/kg/day) as well as a high-fat diet (20% saturated lipids) produces lesions in the prostate gland [26]. The data reveal lesions that occur within a

complex picture of malignancy which warrants further investigation, particularly on the relationship between BPA and estrogen-derived from adipose tissue.

In cancer cell culture models, mitogenic activity of BPA induces the AR mutant, AR T877A, to bind androgen response elements in AR T877A-expressing LNCaP prostate cancer cells while wild type AR fails to respond to BPA exposure [27]. In the same study, BPA induces AR nuclear translocation and increases pRB phosphorylation leading to increased proliferation. This effect was comparable to cells treated with dihydrotestosterone (DHT), the principal hormone that affects the prostate gland. These findings are made particularly important since AR T877A is a mutation found in 12.5% of castration resistant prostate cancers (CRPC), which is the most aggressive and malignant form of PCa [27]. Transcriptome analysis of the same cell line expressing mutant AR T877A led to the identification that BPA down-regulates ERβ mRNA and protein. ERβ is hypothesized to function as anti-proliferative and pro-apoptotic in the prostate. Downregulation would promote PCa growth and/or progression and counteract these effects [28]. *In vitro* investigation using PCa cell lines and immortalized prostate cells found centrosomal abnormalities, microtubule nucleation, and anchorage-independent growth in BPA treated cells [29]. This study also identified higher urinary BPA concentrations in PCa patients compared to healthy controls.

3A. 2. Bisphenol A: Testes

With regards to BPA-induced TCa, Bouskine et al. reported that BPA stimulates cell proliferation through PKA and PKG activation pathway at low doses in the JKT-1 human testicular seminoma cell line [30]. BPA was also found to increase testosterone secretion in MA-10 Leydig cells [31] an observation that may be relevant upon consideration of BPA-induced PCa, especially in early stage disease. Further, in 2017, Liang et al. developed and validated an automated, multi-parametric high-content analysis (HCA) to assess the effects of BPA and its analogs using the C18–4 spermatogonial cell line as a model [32]. The HCA parameters included nuclear morphology, DNA content, cell cycle progression, DNA synthesis, cytoskeleton integrity, and DNA damage responses. While their study focused on assessing novel endpoints for cytotoxicity, attention to characteristics of carcinogenesis show that BPA and its analogs alter cell cycle regulation, cytoskeleton integrity, and induce DNA damage.

Overall, molecular mechanisms of BPA-induced carcinogenesis are lacking in the male GU system, with no studies existing to date in the bladder. BPA exposure at environmentally relevant doses [33] affects the developing prostate gland, often leading to PIN and adenocarcinoma. Studies from various groups throughout the years have reproduced similar results in different animal models, however, there should also be an increased focus on adult animal models. The models developed for the investigation of the prostate could be considered in the investigation of the testes and bladder given the established dosages and bioavailability. The field would benefit from molecular studies evaluating insult to organ health following BPA exposure. This could include the interference of endogenous hormones, examining alterations in feedback loops and/or regulatory hormone axes, as well as or markers of inflammation and immune cell modulation, such as in prostatitis or cystitis,

considered as risk factors in PCa and BlCa development, respectively. Amidst the controversy regarding toxicity and safety of BPA, studies in the male GU system are limited. We believe there is great opportunity for continued exploration in this area. The final conclusions from the CLARITY-BPA studies will help guide future research and direct immediate changes in exposures to the population. It is the onus of the scientific community to continue this investigation in order to gain a better understating of BPA-induced effects on carcinogenesis and cancer hallmarks in the male genitourinary organ.

3B. Diethylstilbestrol

Diethylstilbestrol (DES) is a xenoestrogen used in the 1960s as a growth hormone in the beef and poultry industry. Populations could have been exposed to concentrations of up to 10 ppb within beef and mutton biomass. DES was also given to pregnant women with the belief of a reduced chance of complications resulting in miscarriages and spontaneous abortions. Since then its use has been discontinued because of the association with *in utero* exposure and increased cancer incidence and non-neoplastic abnormalities of the reproductive system [34]. It has been estimated that between 5 and 10 million Americans received DES during pregnancy or were exposed to the drug *in utero* [35]. DES is metabolized by the CYP450 metabolic enzymes which includes epoxidation of the double bonds leading to the formation of epoxide metabolites. These metabolites are able to bind to steroid hormone receptors and are electrophilic to DNA [36]. Further, DES as well as its oxidative metabolites affect microtubule proteins by disrupting the mitotic spindle, which leads to aneuploidy [37]. Aneuploidy and DNA adduct formation correlate with DES-induced cell transformation and are considered to be important in DES-induced carcinogenesis [38]. Despite reports of the association between DES and several cancers, its medicinal use continued through the 1970s and in some cases into the early 1980s [39, 40]. These uses included hormone-replacement therapy, control of menstrual disorders, relief or prevention of postpartum breast engorgement, palliative therapy for prostate and breast cancers, and as a post-coital contraceptive.

3B. 1. Diethylstilbestrol: Prostate and Testes

Early studies in rodent prostate models attribute neonatal DES exposures to increased proliferation along with developmental abnormalities including prostate enlargement and increased duct formation and volume [41–42]. Of note, differential effects were observed in low and high doses of DES exposure, with prostate weight decreasing at higher doses [43–44]. In later years, DES was the first drug used for hormone treatment of PCa and is still used for the treatment of CRPC [45, 46]. Extensive use of DES has since been regulated by the FDA, however, most current exposure is through its oral administration as a drug in clinical trials for the continued treatment of prostate and breast cancers [35]. Nonetheless, widespread use of DES for the treatment of PCa has decreased due to well documented toxicity, including the increased risk of thromboembolism.

Evidence that identifies DES as a risk factor for TCa includes neonatal exposure, epigenetic reprogramming, and conditions that predispose individuals to cancer later in life, such as testicular dysgenesis syndrome [47]. Gill et al., in an early cohort study, found an

association between DES exposure *in utero* and increased incidence of cryptorchidism in men. Cryptorchidism increases testicular cancer risk 5-fold and thus ushered a wave of studies of DES association with TCa risk [48, 49]. To date, prenatal exposure to 100μg/kg DES in animal studies have been used to argue for the role of DES as a testicular carcinogen [50–52]. Nevertheless, inconsistent epidemiological and cohort studies have arrived at opposing or non-significant conclusions in adult human and animal models [48, 53–56]. In a follow-up of prenatal DES-exposed men in 3 US cohorts, inflammation was strongly associated with DES exposure [54]. Although this analysis did not examine cancer as an end point, the implication for a role in cancer cannot be ruled out especially given the role that inflammation plays in cancer development. Further, in a study of over 3600 men with known prenatal DES exposure, a higher rate of TCa was found compared with unexposed men [56]. In addition to these epidemiologic data, DES-exposed mice lineages have been reported to have increased male reproductive tract tumor susceptibility in subsequent generations [57, 58]

Prenatal DES exposure is linked to gross changes in the prostate and frank tumor development in the testes. There are no reports to date of DES-induced PCa; and DES has been widely used to treat PCa. DES for the treatment of PCa however should continue to be monitored not only due to the known risk factors but also because of developing evidence [57, 58] of generational effects on the testes that go beyond the exposed individual. PCa incidence is highest in men older than 50 years old, while TCa is highest in men between the ages 15–35 years old, potentially affecting the progeny of PCa survivors. Additional investigation on the generational effects of DES on TCa development should be considered in treatments and clinical trials. Furthermore, studies on the long-term use of DES should be considered. Overall mechanistic investigations of DES on altered signaling pathways in the prostate, testes, and bladder are lacking. Our search found no animal models or molecular studies of DES as a foundation for BlCa and thus we think a knowledge gap on the effects of DES in the bladder needs to be filled.

3C. Polychlorinated Biphenyls

Polychlorinated Biphenyls (PCBs) were used in the United States for both enclosed applications (such as transformers, capacitors, and heat transfer and hydraulic fluids) and open applications (such as inks, flame retardants, adhesives, carbonless duplicating paper, paints, plasticizers, wire insulators, metal coatings, and pesticide extenders). Since 1974, PCBs have been tightly regulated or banned but are still of public concern because they are considered persistent organic pollutants such that when they enter the environment through continued use or improper disposal, they bioaccumulate due to lipophilicity [59]. Large-scale incidents of soil, livestock, and wastewater contamination are reported in Japan, The Republic of Ireland, and the US [60–62]. A major source of human exposure to PCBs occurs through the consumption of fish from contaminated water bodies, particularly fresh water. For instance, PCBs have frequently been identified at relatively high concentrations in the blood, fat, and milk of native Inuit populations living in Arctic regions, whose diet is high in fish and marine animals [59]. Because PCBs are soluble in fats and oils, the major U.S. commodities in which PCBs have been found are fish, cheese, eggs, and animal feed. PCB residues have also been detected in human milk and fat samples collected from the general

U.S. population. PCBs are hydroxylated by the CYP450 enzymes to produce episodic or persistent congener metabolites. In the blood the most common are 4-OH penta-CB107 and 4-OH hepta-CB187 [63] In the liver and adipose tissues, the most common are 3'-OH haca-CB138 and 4'-OH hepta-CB130 [64]. These metabolites are then oxidized into reactive quinones that act on molecular targets such as forming glutathione conjugates, DNA adducts, and reactive oxygen species. These quinones also bind to proteins, such as steroid hormones. PCBs are glucuronidated by uridine diphosphate glucuronosyle transferase and excreted through the urine and feces. Recent evidence of the adverse effects of PCB exposure in humans and animals is associated with endocrine, dental and neurodevelopmental/reproductive changes, immunological alterations, and cancer [65].

3C. 1. Polychlorinated Biphenyls: Prostate and Testes

Epidemiological and biostatistical analyses suggest positive associations with PCB exposure and the incidence of PCa [66–68]; however, molecular evidence is questionable. In the LNCaP cell culture model, PCB 118 and PCB 153 have biphasic stimulation of androgen-dependent cell proliferation, with induction at lower (0.1–1.0 μg/mL), biologically relevant, concentrations and reduction at higher (10–20 μg/mL) levels [69]. In primary prostate cells from Sprague-Dawley rats, 24 h exposure of the PCB, Aroclor-1254 (0.1–1.0 μg/mL), results in DNA damage in a dose-dependent manner [70]. In the LNCaP cell culture model, Aroclor 1260, 1254, 1248, and 1242 and PCB congeners 42, 128, and 138 antagonize AR transactivation activity in the presence of DHT [71]. A recent microarray study of global gene expression from blood samples of Slovak children exposed to PCB shows differential gene expression in TP53, MYC, BCL2, and LRP12 pathways suggesting strong relationships in potential future tumor incidence, including PCa [72]. A fuller understanding of how PCBs modulate cellular signaling and function in the context of tumorigenesis is needed. In consideration of the effects of PCB on molecular changes in testicular cancer, male offspring of CD-1 mice exposed to aroclor at 50μg/kg/d have no change in testicular weights, yet decreased epididymal weights [73]. Similar to the effects seen in the rete testes of rodents following prenatal exposure of DES [50], adenomatous lesions of the rete testis develop in the South African eland *(Tragelaphus oryx),* a marine mammal shown to have a high PCB body burden [74].

3C. 2. Polychlorinated Biphenyls: Organochlorine Pesticides

A particular class of PCBs that are of certain concern to human health are organochlorine pesticides (OCPs) due to exposure in farming communities and the recalcitrance in natural solids, global transport, distribution, and toxicity [75]. OCPs such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), its ethylene metabolite,1-dichloro-2',2'-bis-p-chlorophenyl-ethylene (*p,p'*-DDE), chlordane, dieldrin, and heptachlor have been linked to carcinogenicity and endocrine disruption in mammals. Concerns over toxicity are exacerbated by OCP hydrophobic characteristics which results in bioaccumulation within fatty tissues [75]. Although pesticide usage and disposal has become highly regulated and monitored through the EPA and FDA, many of these compounds can persist for years affecting human health and development. The metabolic pathway for OCPs includes glutathione S-transferase (GSTs), N-acetyltransferase and cytochrome P450 enzymes [76]. It has been reported that OCPs are incapable of producing DNA damage; however, hepatic

xenobiotic metabolism confers damaging capabilities in the presence of a hypoactive phase II detoxification due to polymorphisms in key genes in carcinogen metabolism, including *CYP1A1, GSTM1, and GSTT1.*

Various epidemiological studies have consistently demonstrated a higher risk of PCa in agricultural populations than in the general population associated with endocrine disrupting pesticides [77]. In an early rodent model assessing OCP effects on adult male rat sexual development, *p,p'*-DDE inhibited androgen-dependent transactivation and acted as an AR antagonist [78]. In the human prostate cancer cell lines LNCaP (AR-dependent) and PC-3 (AR-independent), ERBB-2 kinase is activated by OCP treatment. There is a differential effect on cell proliferation between the cell lines that revolves around AR dependence in response to *p,p'*-DDE due to its role as an AR antagonist [79]. *ERBB2* is a well-studied oncogene in many malignancies that is targeted in the treatment of breast cancer, thus highlighting its importance of activation by OCP treatment. *p,p'*-DDE had no effect on LNCaP cell proliferation, indicating an AR-independent signaling pathway. Another study evaluating human prostate tissues and LNCaP cells reports that *p,p'-DDE* affects 5α-reductase and aromatase, enzymes integral in AR and ERα signaling, respectively, and key players in the development and progression of PCa [80]. Furthermore, in comparing PCa patients with healthy controls, β- and γ- hexachlorohexane as well as p,p'-DDE serum concentrations are significantly increased [81]. This biostatistical study is novel because it also assessed whether there are CYP1A1 polymorphisms in the same patient dataset. Results show there is no significant difference in CYP1A1 polymorphisms among PCa patients in Delhi, India. However, there are mixed results for increase PCa risk due to CYP1A1 polymorphisms in Japan, Turkey, and Northern Delhi [82, 83,84]. The diverging results from these studies highlight the difficulty in capturing geographic differences in doses of EDC exposures as well as genetic differences in EDC metabolism among populations.

To address this pitfall, large-scale case studies of samples from The US Servicemen's Testicular Tumor bank, Environmental and Endocrine Determinants Study, and The Norwegian Janus Serum Bank Cohort. Looking at pre-diagnostic serum samples, the study found significantly elevated p-p'-DDE serum levels in testicular germ cell tumors cases [85, 86]. The persistence of PCB within the environment warrants further investigation on the role of specific PCBs on TCa development. Importantly, p, p'-DDE function as a potent AR antagonist and the impact on the effects on spermatogenesis will increase the understanding of risk factors that lead to TCa. Investigations that consider PCBs in BlCa development are absent beyond the reports of toxic effects of OCPs in its development due to hypoactive phase II detoxification pathway cause by genetic variation in xenobiotic metabolism genes, such as *CYP1A1, GSTM1, GSTT1, GSTP1, NAT1, and NAT2.* A study evaluating north Indian cohorts assessed the pathogenesis of BlCa with the association of *GSTT1 and GSTM1* gene polymorphisms. They discovered null deletions are significantly higher in BlCa cases. Furthermore, BlCa patients had significantly higher plasma levels of OCP residues compared to controls, which significantly correlates with *GSTM1 and GSTT1* deletion [76].

Although PCBs have largely been banned and/or tightly regulated in most industrialized societies, their hydrophobic chemistry has caused bioaccumulation that has persisted in the

environment. Furthermore, in tropical climates that rely heavily on OCPs for mosquito vector control or farming in communities, exposure remains a large risk factor for a myriad of health complications. Considering the different PCP congeners and metabolites, animal models and cell models, as well as biological endpoints, the field is ripe for investigation. Intriguingly, because PCPs are persistent in the environment, there may be animal models readily available for investigation such as seen in the study with the South African eland [74]. Overall, when it comes to PCP and OCP research in molecular carcinogenesis, there is great need to support epidemiological and biostatistical datasets.

4. Conclusions and challenges

The present number of reports describing genotypes, phenotypes, and other biological endpoints of molecular carcinogenesis linking EDC exposures to the etiology of male urological malignancies is overwhelmingly low. The molecular effects of BPA are the most studied and the prostate has been the most studied organ in EDC exposure overall. There is an immense knowledge gap regarding the effects of EDCs on the testes and the urinary bladder (Table 3).

While past findings of the effects of EDCs on human health have directed life-saving outcomes, such as the link between DES exposure and vaginal adenocarcinoma, the small number of overall research reports on EDCs in the GU field is a limitation. This affects the ability to develop testable hypotheses, draw conclusions, and provide preventative measures in potentially modifiable conditions. Obstacles in successful elucidation of the mechanisms of EDC-induced malignancy include: 1) Differing biological effects of chemicals and their metabolites as well as pathological effects that depend on length and dose of exposures [87]. This is especially relevant when considering low dose exposures to EDCs can have a large biological impact [88]. 2) The estimation of exposure lengths and concentrations in populations worldwide is difficult to capture and recapitulate in the laboratory setting. 3) Animal models of particular EDC-induced cancers may not exist due to differences in metabolism among species; and 4) resistance from governmental, industrial, and private interests due to the potential impact on infrastructure and consumerism.

5. Future Directions

Although recent studies in animal models were geared towards perceived biologically relevant doses, these data may underestimate EDC bioactivity. Furthermore, there is room to explore the various metabolites of EDCs as well as genetic variations of EDC metabolism. More studies considering high and low dose exposures will distinguish thresholds and allow the development of confident TDIs. This will also allow the development of innovative treatments and techniques to safely use EDCs in cancer treatment, such as the use of DES in PCa.

The development of humanized animal models would assist to resolve all three of the obstacles described above. In a relevant development, the most recent report of the Endocrine Society encourages the use of rodent models as described by the two-year bioassay protocols defined by the National Toxicological Program [68]. Furthermore, ever-

emerging computational biology will allow the refinement of computer models that could calculate risk based on pertinent considerations, including but not limited to, length and dose of exposures, concurrent exposures, diverse ethnic polymorphisms in metabolic genes, as well as age and gender. Tissue models in an ex-vivo or co-culture system can also be developed to capture the interaction of different cell types and molecules within the microenvironment following EDC exposure.

Future studies that evaluate the role of EDCs in urological malignancies are appropriate within the realms of either the somatic mutation theory or developmental origins of disease. Examples of mechanistic studies include the effects of EDCs on neuroendocrine control of the urogenital system, the effects of EDCs on hormone receptors, gender-specific effects of EDCs, the molecular outcomes of concurrent exposures, as well as effects on transcriptional regulation of oncogenes and tumor suppressors. The potential for discovery is limitless. Until then, prevalent and persistent unknowns make it difficult to develop preventive measures and modify risk factors.

Increased industrialization directs EDC pervasiveness in food, water, and household products. Increased research in the field has the potential to understand biological consequences of EDC exposure in humans, animals and vegetation. In addition to cancer prevention, uncovering the mechanistic effects of EDCs will aid in prevention of other diseases that are known to involve EDCs. Overall, continued EDC research will increase awareness and understanding of our natural and manmade environment and promote disease prevention.

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Abbreviations

EDC	Environmental Endocrine Disruptors
EPA	Environmental Protection Agency
GU	Genitourinary
PCa	Prostate cancer
BlCa	Bladder cancer
TCa	Testicular cancer
BPA	Bisphenol A
PIN	Prostate intraepithelial neoplasia
PDE4D4	Phosphodiesterase Type 4 Variant 4
AR	Androgen receptor

DHT	Dihydrotesterone
CRPC	Castration resistant prostate cancer
DES	Diethystilbestrol
ER	estrogen receptor
PSA	prostate specific antigen
RWPE-1	non-malignant transformed prostate epithelial cells
PCBs	polychlorinated biphenyls
OCPs	organochlorine pesticides
GSTs	glutathione S-transferase
p, p'-DDE	1,1-dichloro-2',2'-bis-p-chlorophenyl-ethylene

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Table 1

Reported molecular targets of environmental endocrine disruptors.

Environmental endocrine disruptors have a broad range of effects on the human body. The mechanisms converge and encompass critical molecular mechanisms, including, nuclear receptors, non-nuclear receptors, the neuroendocrine axes, enzymatic pathways, cell signaling pathways, and gene expression mechanisms.

Nuclear Receptors	Non-nuclear Receptors	Neuroendocrine Axes	Enzymatic Pathways	Cell Signaling Pathways	Gene Expression/Epigenetics
Steroid hormone receptors [1,15,27,28,36,69,71,78,79]	Membrane receptors [15–17,90]	Hypothalamic-Pituitary [93,94]	Organification of iodine by thyroperoxidase [95]	Cerebellar motor behavior [99]	Transcription factors: AP-1, STATS, NFκB and Sp1 [100]
Peroxisome proliferator-activated receptor (PPAR) γ [89]	Sodium/iodine symporter on thyrocytes [91]	Hypothalamic-Pituitary-Thyroid [15,93]	T_4 conversion to T_3 by deiodinase [96]	MAPK signaling cascade [15,30,98]	DNA methylation [19,22,23]
Aryl hydrocarbon receptor [1]	Receptors of neurotransmitter neuronal cells: noradrenergic, serotonergic, dopaminergic [92]	Hypothalamic-Pituitary-Adrenal [94]	Lipogenesis, lipolysis, and adipogenesis [26,97]	cAMP and adenylate cyclase, calcium, PI3K, PKB, Src [19]	Histone acetylation [101]
			Endothelial nitric oxide synthase [98]		DNA replication [29,32,37,38,69,70,72]
			Steroidogenesis [24,25,31,80]		

Table 2:

Molecular effects of EDCs in the prostate gland, testes, and urinary bladder.

Current studies report the molecular effects and biological endpoints of EDC exposures including enzyme activity, gene regulation, DNA damage and repair, as well as cell proliferation mechanisms. These effects are due to exposures at low and high doses in cell culture and animal models.

	Prostate Gland	Testes	Urinary Bladder
Gene Regulation/Epigenetics	BPA OCP	DES	OCP
Enzyme Activity	BPA DES OCP		
DNA Damage	PCB	BPA	
Cell Cycle/Cell Proliferation	BPA DES	BPA	
Anatomic Features	BPA DES	DES (Frank tumors) PCB	

Table 3:

Specific Molecular aberrations of EDCs in the prostate gland, testes, and urinary bladder.

Reported effects on molecular mechanisms, including EDC targets on molecules in transcription, epigenetics, and cell cycle regulation. There is also evidence of DNA damage as a result of metabolic gene polymorphisms and null deletions.

EDC	MOLECULAR ABERRATION	Mimics/Disrupts
Prostate		
BPA	Altered cAMP breakdown	Mimics AR
	AR T877A mutant	Mimics AR
	DNA methylome changes	-
	Aberrant mitosis (centrosomal abnormalities, microtubule nucleation)	-
	Anchorage-independent cell growth	-
	Altered aromatase and 5α-reductase activity	Mimics AR and ER
PCBs	Antagonize AR transactivation activity	Disrupts
	Activated ERBB-2 kinase (OCPs)	Mimics
	Polymorphisms in *CYP1A1* (OCPs)	-
	Altered aromatase and 5α-reductase signaling	Disrupts
	GSTT1/GSTM1 null deletions (OCPs)	-
Testes		
BPA	PKA and PKG mediated cell proliferation	Mimics AR
	Increased testosterone secretion	Mimics AR
	Altered cytoskeleton integrity	-
Bladder		
PCBs	GSTT1/GSTM1 null deletions (OCPs)	-