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Bridging epidemiology and model organisms to increase understanding of endocrine disrupting chemicals and human health effects☆

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ABSTRACT

Concerning temporal trends in human reproductive health has prompted concern about the role of environmentally mediated risk factors. The population is exposed to chemicals present in air, water, food and in a variety of consumer and personal care products, subsequently multiple chemicals are found human populations around the globe. Recent reviews find that endocrine disrupting chemicals (EDCs) can adversely affect reproductive and developmental health. However, there are still many knowledge gaps. This paper reviews some of the key scientific concepts relevant to integrating information from human epidemiologic and model organisms to understand the relationship between EDC exposure and adverse human health effects. Additionally, areas of new insights which influence the interpretation of the science are briefly reviewed, including: enhanced understanding of toxicity pathways; importance of timing of exposure; contribution of multiple chemical exposures; and low dose effects. Two cases are presented, thyroid disrupting chemicals and anti-androgens chemicals, which illustrate how our knowledge of the relationship between EDCs and adverse human health effects is strengthened and data gaps reduced when we integrate findings from animal and human studies.

1. Introduction

Concerning temporal trends in human reproductive health has prompted concern about the role of environmentally mediated risk factors. Studies report increases in reproductive diseases and decline in reproductive function since the mid-20th century among certain locations and populations (primarily in the developed world), previously reviewed and illustrated in Table 1 [1].

The relatively short time frame over which decline in reproductive health and function has been observed cannot be explained by genetic changes. Environmental chemicals have been identified as one of the potential risk factors that may be contributing to observed changes in reproductive health [2–4]. Over roughly the same period, manufacture and use of both natural and synthetic chemicals has increased by over 20 fold [5]. In the US, there are approximately 87,000 chemical substances registered for use in commerce as of 2006, and about 3000 chemicals manufactured or imported in excess of 1 million pounds each [6].

The population is exposed to chemicals present in air, water, food and in a variety of consumer and personal care products [48–51]. In the United States, nationally representative samples of population through the National Health and Nutrition Examination Survey find that every individual has measured levels of multiple environmental chemicals in his/her body [7,8]. There are similar findings from studies in Europe [9], and populations in the Arctic far from pollution sources [10]. Thus it is expected that all human populations are exposed to some level of synthetic chemicals.

Previous studies have demonstrated environmental chemicals can adversely impact human health. Poisoning incidents with mercury in Japan and polychlorinated biphenyl (PCB) in Taiwan, produced neurological, reproductive, and developmental effects, even when the mother was asymptomatic [11–13]. Occupational exposures to 1,2-dibromo-3-chloropropane (DBCP), produced male infertility [14]. Science has evolved to study health effects of lower level exposures among the population. For example, reviews of the epidemiological evidence find that contemporary exposures to PCBs are associated with a decrease in semen quality, specifically reduced sperm motility [2] and prenatal exposures to methylmercury and PCBs at contemporary concentrations can increase risk of neurological deficits in children [15–18].

Environmental contaminants can adversely affect reproductive health through diverse biological mechanisms. Historically, much of the scientific inquiry focused on genotoxic or mutagenic chemicals and cancer effects [19,20]. Since the latter part of the 20th century there has increased emphasis on an important class of chemicals called endocrine disrupting chemicals (EDCs) that interfere with the production, release, transport, metabolism, binding,

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Table 1
Examples of recent trends in select reproductive disease, disorders and function[a].

Reproductive diseases/disorders	Increase	Period	Location	Reference
Testicular cancer	1–6%	1953–1999	Europe	[115]
Testicular cancer	60%	1973–2003	USA	[116]
Certain childhood cancers	20–24%	1976–2005	USA	[117]
Autism	57%	2002–2006	USA	[118]
Attention deficit hyperactivity disorder	3% per year	1997–2006	USA	[119]
Birth defects				
Cryptorchidism	200%	1970–1993	USA	[120]
Gastroschisis	300%	1978–2005	California	[121]
Congenital hypothyroidism	138%	1987–2003	New York	[122]

Reproductive function		Time	Location	Reference
Reported difficulty conceiving and maintaining pregnancy				
All ages	60% more women	1982; 2002	USA	[123,124]
<25 years old	200% more women	1982; 2002	USA	[123,124]
Prematurity	2.9% shorter gestation	1992–2002	USA	[125]
Pre-eclampsia	19–36%	1968-2002	Norway	[126]
Gestational diabetes	122%	1989-2004	USA	[127]
Premature puberty				
Age at onset of breast development	1–2 years younger	1940–1994	USA, Denmark	[3,128]
Age at onset of menstruation	2.5–4 months younger	1940–1994	USA	[3]
Sperm count	~1% decline per year	1931–1994	Western countries	[129,130]
Serum testosterone	1% decline per year	1987–2004	Boston, USA	[135]

[a] Updated from Woodruff et al. [1].

action, or elimination of natural hormones in the body and are responsible for maintenance of homeostasis and regulation of developmental processes and their potential effects on reproductive and developmental effects [2]. Recent reviews and scientific consensus statements find that "the evidence for adverse reproductive outcomes (infertility, cancers, malformations) from exposure to endocrine disrupting chemicals is strong, and there is mounting evidence for effects on other endocrine systems, including thyroid, neuroendocrine, obesity and metabolism, and insulin and glucose homeostasis." [2].

However, there are still many gaps in our understanding of the relationship between EDC exposures and reproductive and developmental effects. First, the number of chemicals that have been evaluated for effects on human health remains limited [6]. Second, many reproductive and developmental health conditions, such as female reproductive effects (e.g. fibroids, endometriosis), male reproductive effects (e.g. cryptorchidism, prostate cancer), and childhood diseases (e.g. obesity, cancer), have not been fully evaluated for the likely contribution of EDCs as a risk factor. As new efforts are initiated to address knowledge gaps the relationship between EDCs and reproductive and development outcomes, understanding how findings from human and model organisms can each contribute to filling the gaps is critical. Findings from human observational studies provide direct evidence of the relationship between exposure to environmental chemicals and subsequent adverse health effects. However, limitations in the epidemiologic data, both scientific and ethical, require the use of findings from mammalian and other model organisms to compliment human epidemiologic evidence. This paper reviews some of the key scientific concepts relevant to integrating information from human epidemiologic and model organisms to understand the relationship between EDC exposure and adverse human health effects. This is followed by two cases studies: first on thyroid disrupting chemicals; and second on anti-androgens chemicals, illustrating how the interplay between the findings from two disciplines can enhance our understanding of the relationship between exposure and disease.

2. Key concepts and new science

2.1. Sources of scientific information

Our understanding of potentially harmful effects of exposure to environmental contaminants comes from a variety of sources

Table 2
Comparing experimental features of human epidemiology studies and experimental animal studies.

Human epidemiologic data	Experimental animal data
Exposure issues	
Exposure to chemical(s) may be unclear or difficult to estimate, individual may not remember what they were exposed to or unaware of exposure	Controlled dosage of chemical(s)
Reflects real world setting of multiple exposures, but can make it challenging for interpreting effects of individual chemical	Often only exposed to individual chemical, does not account for exposure to other chemicals that may affect same adverse health outcome
Duration of exposure often unknown	Controlled duration
Exposure may cover multiple windows of susceptibility or may not include specific exposure timing for critical windows	Controlled exposure can be applied for known or suspected periods of susceptibility
Experimental design	
Generally make use of existing data, which often cross-sectional and/or retrospective	Study design is experimental
Confounding factors may be present (diet, disease, smoking, etc.)	Confounding factors are reduced through experimental design (controlled exposures and diet) and use of healthy animals
Genetic variability	Known genetics: selection of homogeneous or heterogeneous strains
May have indirect determination of outcomes (endpoints) (e.g. from birth certificates, medical records, for example)	Direct endpoints studied
Interpretation and use of results	
No need for extrapolation to human populations and usually not for high to low dose	Need to extrapolate to humans and often from high to low dose
Human studies can take many years	Studies can be performed in short periods of time, usually less than 2 years

including wildlife life studies, *in vitro* and *in vivo* toxicology studies, epidemiologic studies, and clinical evidence. The most extensive information comes from animal studies, which are a preferable method for assessing potential adverse human effects and for developing strategies for prevention of harmful exposures. Environmental contaminants are not intended for human use, and it is unethical to knowingly expose humans to these chemicals under experimental conditions to assess for harmful effects.

2.1.1. The role of model organisms

Animals have long been used to understand the effects of chemical exposure on human reproduction and development [21]. One of the first reproductive and developmental studies, from 1919, found alcohol affected fetal development in rats before it was confirmed in human studies [22,23]. The reliability of experimental animal data for reproductive and developmental health has been well established and presently, there is no example of a chemical agent that has adversely affected human reproduction or development but has not caused the same or similar adverse effects in animal models [24]. Model organisms studies have many advantages, including ability to control the experimental setting to enhance ability to observe chemical effects, and shorter time from exposure to disease [25]. Multiple reviews have concluded that there is concordance of developmental and reproductive effects and that humans are as sensitive or more sensitive than the most sensitive animal species [23,24,26–29]. Given that there is general conservation of biologic function across animal species, including humans; animal studies provide important insights into potential human harm [30].

However, limitations in the design of traditional toxicological studies have limited their utility for studying reproductive or developmental outcomes. These include: use of insensitive strains; exposures that do not cover sensitive periods of development; exposures to single chemicals at high doses rather than the mixture of low doses of chemicals experienced by the public; and a focus on overt disease endpoints. While animal studies continue to provide important information to predict human harm, epidemiologic and clinical studies provide complimentary sources of information.

2.1.2. The role of epidemiology

Human observational studies of environmental chemicals provide the most direct evidence of the relationship between exposure and increased risk of adverse health outcomes, and are often the basis of assessments about harmful effects of EDCs. Studies are typically designed to evaluate whether the change in the risk factor of interest, or the chemical exposure, is related to the change in the incidence or prevalence of the disease of study, while at the same time accounting for factors that may influence that relationship. Model animal studies tend to focus their methodological scope by reducing variability in potential influential factors to increase the ability to detect presence of an effect from chemical exposure, while human observational studies necessarily include the factors which can influence the exposure/effect relationship as they are often difficult to control. These include pre-existing exposure to other potentially influentially chemicals, biological variability (e.g. disease status, age, gender), and typically lower levels of exposure. This makes the interpretation of the results of observational human studies challenging because these factors that influence risk (e.g. age, disease status, co-exposures), can obscure the signal for a particular chemical/outcome relationship. Features of model organisms and epidemiology are illustrated in Table 2.

2.2. Emerging scientific themes and their implications

Since the latter part of the 20th century, the evolution of our understanding about the role of EDCs in human health has been accompanied by increasing insights into additional factors which influence the relationship between chemical exposure and risk of adverse health effects. These include: enhanced understanding of toxicity pathways; importance of timing of exposure; contribution of multiple chemical exposures; and low dose effects. The growth in the science in each of these areas is influencing study designs in epidemiology and model organisms, the interpretation of the data from each, and the need to integrate knowledge from both the fields. Each of these areas and their contribution to the field is discussed below.

2.2.1. Biological pathways

Advances in molecular biology, computational biology and information sciences is transforming our ability to interrogate and understand disease etiology on multiple levels, which in turn requires advancing our approaches to assessing potential risks from chemical exposures [31,32]. We are faced with expanding information at the genetic level, such as through genome wide association studies [33], epigenetic assessments [34], and metabolomics [35], providing unprecedented information about the pathways from exposure to overt disease. Concurrently, the technological capacity to test multiple chemicals and their effects on biological pathways rapidly through high throughput vitro assays expands the amount

of knowledge available for interpretation [36]. The advancement of scientific ability to evaluate early perturbations in the disease process increases the opportunity to identify potentially harmful chemicals using early biological markers of disease [4], and is a feature which can be integrated across the interpretation data from human and model organism studies.

2.2.2. Periods of susceptibility

Human development is vulnerable to biological disruption particularly when these changes occur during critical windows of development, which typically occur peri-conceptually, during pregnancy, infancy, childhood, puberty, pregnancy and lactation. Perturbations from chemical exposures can lead to important functional deficits and increased risks of disease and disability in infants, children and across the entire span of human life [4,30,37]. The prenatal period has become a particular area of focus of environmental chemical exposure for two primary reasons. First, over the past 60 years it has become clear that the placenta does not protect the fetus from damaging chemicals [38], that chemicals can be found in the fetus, and in some cases may be biomagnified. For example, analysis of second trimester amniotic fluid samples from 51 women found the presence of at least one environmental contaminant [39], and for mercury, fetal exposures to environmental contaminants may be higher than maternal [40–42]. Second, while the effects of prenatal exposure on immediate outcomes, such as birth defects or childhood illness has been relatively well known [15], newer science find that *in utero* disturbances from the external environment can increase risk of adult disease (in particular the central nervous system, the cardiovascular system, the endocrine system and the immune system), referred to as the Developmental Basis of Adult Disease/Dysfunction [2].

2.2.3. Chemical background, exposure to mixtures and biomonitoring

Numerous animal studies now find that co-exposures to chemicals can enhance risks of adverse health effects compared to risks from exposure to individual chemicals, even if the chemicals act through different mechanisms of action [43–46]. The National Academy of Sciences recommends that chemical exposures that can affect the same common adverse outcome should be considered together, and in many cases are additive [47].

Analyses of CDC population-based biomonitoring data among pregnant women in the U.S. finds virtually all pregnant women have body burdens of a number of EDCs in their body include certain pesticides, perchlorate, Bisphenol-A (BPA), phthalates, perfluorochemicals (PFCs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Table 3).

Table 3
Percentage of U.S. pregnant women with select EDCs measured in their body.*

Chemical analyte	Percent of U.S. pregnant women with detectable levels of the analyte
PFCs (serum; μg/L) (*N* = 76)	
PFOA	99
PFOS	99
PBDEs (serum; ng/g lipid) (*N* = 75)	
BDE-47	99
BDE-99	87
BDE-100	99
BDE-153	100
PCBs (serum; ng/g lipid) (*N* = 75)	
PCB-118	100
PCB-138 and 158	100
PCB-153	100
PCB-180	96
Organochlorine (OC) pesticides (serum; ng/g lipid) (*N* = 71)	
DDT	62
DDE	100
Hexachlorobenzene	100
Environmental phenols (urine; μg/L) (*N* = 86)	
Bisphenol-A	96
Triclosan	87
Benzophenone-3	100
Phthalates (urine; μg/L) (*N* = 91)	
Mono-n-butyl phthalate (MnBP)	99
Mono-ethyl phthalate (MEP)	100
Mono-benzyl phthalate (MBzP)	100
Mono-isobutyl phthalate (MiBP)	99
Perchlorate (urine; μg/L) (*N* = 89)	
Perchlorate	100

* Based on analysis of representative sample of U.S. population by the U.S. Centers for Disease Control and Prevention, National Health and Nutrition Examination Survey 2003–2004 adapted from Woodruff et al. [133].

2.2.4. Biological background

Background health status, as influenced by age, pre-existing disease, genetics, and other factors, can influence the effect of chemical exposure on subsequent health risks. For example, individuals with preexisting immune suppression, such as organ transplant patients, those who are HIV positive, and those at early or late lifestages, might experience disproportionately higher risk of adverse health effects due to chemical exposure compared to more healthy individuals. A vast number of biological processes that are ongoing throughout a lifetime can contribute to disease etiology. For example, neurological deficits and cancer, are relatively prevalent in the population, and have ongoing biological process that contribute independently to disease etiology, and EDC exposure could add to these ongoing biological processes [52].

2.2.5. Multiple mechanisms of action

Exposure to a chemical can influence disease processes through multiple mechanisms of action. Hence, studies that focus on a particular pathway or individual biological event may provide an incomplete picture of the health risks produced by a particular chemical [53].

2.2.6. Effects at low doses

Traditionally, the evaluation of noncancer effects from chemical assumes that there is an exposure below which exposures are anticipated to not contribute to increase risk of disease (referred to as a "threshold"). However, scientific evidence now finds that adverse health effects can occur from exposures at common population levels (e.g. lead and mercury), and threshold cannot be identified in the human population. Further, background chemical exposures, pre-existing vulnerabilities in the population, such as from age and pre-existing disease, can contribute to population background risks such that practically, a threshold will not exist in the population and we expect risks even at low exposures to individual chemicals [52].

2.3. Implications and case studies

Our increasing appreciation of the influence of biological background (i.e. age and disease status) and chemical background to EDC and disease etiology comes from integrating findings from human and model organisms studies. In turn, this base of knowledge guides the interpretation of insights from studies from both disciplines. The following case studies illustrate the importance of each and how scientific concepts aid in interpreting and expanding our knowledge base.

2.3.1. Thyroid hormone perturbations and neurodevelopmental outcomes

This case study of thyroid hormone (TH) perturbations during pregnancy and neurological outcomes illustrates how human

Table 4
Classes, mechanisms of action, and effects of thyroid disrupting chemicals on thyroid hormone homeostasis[a].

Class	Mechanism	Effects on THs	Chemicals
Iodine transport	Competition/block of NIS	Decreased thyroidal synthesis of T3 and T4	Perchlorate, chlorate, bromate, nitrates, thiocyanate
Synthesis inhibitors	Inhibition of TPO	Decreased thyroidal synthesis of T3 and T4	Methimazole, propylthiourea, amitrole, mancozeb, soy isoflavones, benzophenone 2, 1-methyl-3-propyl-imidazole-2-thione
Transport disruption	Altered binding to serum transport proteins	Unknown	Hydroxyl-PCBs, EMD 49209; pentachlorophenol
Enhanced hepatic catabolism	Upregulation of glucuronylsyltransferases or sulfotransferases (via CAR/PRX or AhR)	Increased biliary elimination of T3 and T4	Acetochlor, phenobarbital, 3-methylcolanthrene, PCBs, 1-methyl-3-propyl-imidazole-2-thione
Enhanced cellular transport	Upregulation of OATPs or MCT transporters via CAR/PRX or AhR	Increased biliary elimination of T3 and T4	TCPOBOP, pregnenolone-16α-carbonitrile, TCDD, rifampicin, phenobarbital, oltipraz
Sulfo-transferases	Inhibition of sulfotransferases (SULTs)	Decrease sulfation of THs	Hydroxlyated PCBs, triclosan, pentachlorophenol
Deiodinases	Inhibition or upregulation of deiodinases	Decreased peripheral synthesis of T3	FD&C Red Dye #3, propylthiouracil, PCBs, octyl-methoxycinnamate
TR agonists and antagonists	Direct or indirect alterations in TR-TRE binding	Altered activation of TH dependent gene transcription	TetrabromoBisphenol A, Bisphenol-A, HydroxyPCBs

[a] Reprinted from Crofton [43].

studies provided initial evidence of the link between exposure and outcome that was later enhanced by studies in model organisms.

2.3.1.1. Thyroid hormone overview. THs, thyroxine (T_4) and triiodothyrine (T_3), are essential to neurological development. Insufficient levels of TH during development can lead to mild to severe cognitive impairment, neurobehavioral disorders, hypomyelination and attendant physical impairments [54,55].

First insights into effects of TH decrements during pregnancy on neurological development comes from studies during the first half of the 20th century of human populations with severe endemic goiter, caused by insufficient TH, where children were found to be increased risk of mental retardation and other neurocognitive deficits [56]. While clinically relevant decrements in T_4 during pregnancy were recognized as harmful, later epidemiological studies observed neurobehavioral delays in children born to women with mild to moderately low levels of T_4, not indicative of maternal hypothyroidism, during the first trimester of pregnancy [57–59] [57,59–61]. Other epidemiologic studies found that small deficits in circulating levels of TH are associated with decreased cognitive performance at various times during development and adulthood [62–68].

2.3.1.1.1. Period of susceptibility. The fetal thyroid gland does not begin to concentrate iodide until the 12th gestational week [69] and does not produce significant quantities of TH until about week 20 [69]. Thus, until about the 20th week the fetus is entirely dependent on maternal THs [70], and T4 decrements during this time period can have a greater impact of subsequent neurological risks.

While human studies have shown the importance of the relationship between maternal TH and neurocognitive development in the child, mechanistic studies of the role of chemical exposures in TH changes has been an important complement to the field. They have enhanced our understanding of the mechanisms involved, the changing TH levels during fetal development, role of multiple chemical exposures, and the importance of different THs to neurodevelopment.

For example, the effects of TH on the developing brain are related to serum concentrations of T_4 in laboratory animal studies, with animal studies also finding that the developing brain is sensitive to small reductions in serum TH [71–79]. For example, declining serum T_4 has been shown to be associated with linear declines in the number of oligodendrocytes and increases in the number of astrocytes in white matter of rat pups [80], and effects on the developing hippocampus [77].

2.3.1.1.2. Multiple chemical exposures. A number of environmental chemicals are capable of disrupting TH levels, often through different mechanisms (illustrated in Table 4) [81,82]. For example, perchlorate inhibits the uptake of iodine, resulting in decreased synthesis of TH. PCBs and the pesticide acetochlor activate enzymes in the liver that increase excretion of TH, thus reducing circulating levels of TH [83]. Crofton et al. evaluated rats exposed to a mixture of 18 TDCs (dioxins, dibenzofurans and PCBs), at doses comparable to human exposures, for effects on serum T_4 [43]. Components of this mixture affect T_4 through two different mechanisms of action: the dioxins, dibenzofurans and dioxin-like PCBs activate one set of liver enzymes, and the non-dioxin-like PCBs activate a separate set of liver enzymes that metabolize THs. Crofton et al. found the mixture had a dose-additive effect on T_4 at environmentally-relevant doses and a 2–3 fold greater than dose-additive effect on T_4 at higher doses [43], demonstrating that exposures to TDCs acting on different mechanisms can have cumulative effects.

2.3.1.1.3. Metrics of TH disruption. Clinical sciences has relied on changes in TSH to indicate thyroid related condition [84]. However, there are situations where serum T_4 and T_3 levels may not be associated with serum TSH as they are under normal conditions, such as in the case of liver disease, or if there are defects in the proteins responsible for transporting T_4 and T_3 in the circulation or in the receptors mediating negative feedback of T_4 on TSH [85,86]. Likewise, there are chemicals, such as PCBs, that can cause a decrease in serum total and free T_4 in animals without causing a concomitant increase in serum TSH [87,88].

2.3.1.1.4. Biological background. Significant portions of the population are already at risk from additional T_4 decrements. About one third of U.S. women, including pregnant women, have low iodine intake (iodine is required for TH synthesis) [89]). In addition, between 1999 and 2002, 7.3% of the U.S. population aged 12 years and older reported that they had thyroid disease or were taking thyroid medication [90]. In addition, among women receiving TH replacement medication for treatment of hypothyroidism, 14% had TSH and T_4 measurements indicating continued hypothyroidism [90]. Pregnancy causes an increased demand on the thyroid gland and hypothyroidism is more than twice as common among pregnant women than among non-pregnant women ages 12–49 [90].

2.3.1.2. Conclusions regarding thyroid data. A number of environmental chemicals are capable of disrupting TH levels, including decreasing circulating levels of T_4. Compensatory mechanisms are likely insufficient to counteract the potential adverse health effects

of these T_4 decrements, and thus the fetus is at increased risk for neurological effects from exposure to low levels of TDCs. This conclusion is supported by complimentary findings from the animal and human literature. First, findings in both animals and humans indicate that even small T_4 decrements can have adverse neurodevelopmental consequences in the offspring. Second, fetuses and infants do not have stored TH, and thus have limited capacity to respond to TH decrements during critical stages of development. Third, human evidence find a substantial prevalence of TH and iodine insufficiency in the population of U.S. women, putting them an increased risk of exposure to chemicals that affect TH levels. Fourth, animal studies find that exposure to TDCs can act together to affect TH levels and that human studies show people are currently exposed to multiple TDCs.

2.3.2. Anti-androgens and male reproductive development

A number of different chemicals can affect androgen action. Phthalates are a relatively well characterized class of chemicals in this group and provide a case example of how model organisms played a pivotal role in our understanding of the effects on anti-androgenic substances on male reproductive development that lead to complimentary human studies, significantly increasing our overall knowledge in this field.

Phthalates are a group of industrial chemicals used as plasticizers, and as solubilizing and stabilizing agents. They are found in numerous products including personal care products, medical devices, various plastics include flooring, polyvinyl chloride type plastics and tubings [8]. It was discovered in 2000 that phthalates were also ubiquitously measured in people through reports from the first CDC national biomonitoring report and in publication [91].

2.3.2.1. Effects of exposure to anti-androgens during fetal life. Proper male reproductive tissue development is dependent on a transient peak in testosterone levels during fetal development. Disruption to androgen activity during this critical developmental window can result in a number of male reproductive tract abnormalities, including retained female structures, such as nipples (in animals), and malformations of male reproductive structures, such as hypospadias (an abnormal location of the urethral opening) and cryptorchidism [53].

2.3.2.2. Evolution of our understanding of critical windows and anti-androgens. Early studies of phthalates focused on adult exposures and primarily effects other than reproductive, though some standard reproductive studies noted effects on male rat testicles [92]. Differential effects by age were noted in early studies, with early ages being more susceptible, though it was not until later studies when effects specific to the fetal exposures were identified through multigeneration study of DBP in the rat [93]. Follow up studies in rats identified a critical window of exposure during fetal developmental [94–98]. Additionally, studies identified a sensitive period of exposure during gestational days 15–17 for dibutyl phthalate exposures in the rat, about gestational weeks 8–14 in humans [99].

Phthalates appear to interfere with androgen activity by inhibiting testosterone synthesis. Specifically, phthalates with four to six carbon side chains interfere with the production of testosterone by inhibiting the uptake of cholesterol into the mitochondria by Steroidogenic Acute Regulatory protein (StAR) protein and by inhibiting some, but not all, of the enzymes in the steroidogenic pathway [100]. These phthalates have been most consistently shown to reduce testosterone production in animal research to date, though other phthalates may also be important [101,102].

Studies in rats have demonstrated that a phthalate-induced decrease in testosterone predictably results in a syndrome of anti-androgenic reproductive abnormalities characterized by: malformations of the epididymis, vas deferens, seminal vesicles, prostate, hypospadias, cryptorchidism and testicular injury; permanent changes (feminization) in the retention of nipples and areolae (sexually dimorphic structures in rodents); and demasculinization of the growth of the perineum, resulting in a reduced anogenital distance [98]. This constellation of effects has been referred to as the "phthalate syndrome" and, as with other anti-androgenic chemicals, the severity of effects increases with the dose [99].

The spectrum of effects seen in phthalate syndrome parallels a spectrum of human diseases that has been termed "testicular dysgenesis syndrome" (TDS): infertility, cryptorchidism, hypospadias, and testicular cancer, which has been hypothesized to have a fetal origin associated with male development of the testis [103].

2.3.2.3. Role of human observational studies. In 2000, with publication of the first CDC biomonitoring results in the peer reviewed literature, Blount et al. showed that there was widespread exposure in the US population to a number of different phthalates, finding metabolites of DBP, DEP and BzBP in over 95% of the population [91]. They also found that women of reproductive age had significantly higher levels of metabolites of DBP than other age and gender groups. While phthalates had been measured in the various species since the 1970s, including deep-sea jellyfish [104], fish caught in various parts of North America [105], and bovine tissue [106], this was the first measurements in humans of wide spread common exposure. Further, the higher exposures among women of reproductive age combined with findings in animals suggested that male reproductive development could be at risk.

Given ubiquitous exposure in the human population, identified male reproductive effects, and analytic ability to measure body burdens, human epidemiologic studies could evaluate potential health effects at exposure levels experienced by the general population, which are lower than the doses in the animal studies. Several epidemiological studies have evaluated the relationship between adult exposures and male reproductive effects, in particular semen quality – with some studies finding an association between increased phthalate levels and decreases in semen quality in males [107] (though not in every study [108]).

Other human studies evaluated prenatal exposures, previously identified by animal studies as a sensitive period for exposure. In particular, the study by Swan et al. found associations between certain prenatal phthalate exposure and male developmental reproductive outcomes, in particular shortened ano-genital distance [109]. This study was notable because it used a marker of male reproductive effects reflecting feminization that was common in toxicological studies, but not applied in epidemiologic studies – ano-genital distance. Subsequent human studies have observed ano-genital distance to be sexual dimorphic and reflective of penile length and growth in males [110,111]. This novel application was possible only by cross fertilization across model organisms and human observation studies. Other studies also identified effects from developmental exposures including changes in reproductive hormone levels in male infants exposed to phthalates in breast milk [112].

2.3.2.4. Exposure to mixtures of androgen receptor antagonists and androgen synthesis disruptors. Studies find that exposure to a mixture of chemicals with both similar and different mechanisms of action results in dose-additive effects [46,113]. Rats exposed to a mixture of androgen receptor antagonists, vinclozolin, procymidone, and flutamide, at doses that would not have caused hypospadias alone, resulted in over 50% of animals with hypospadias [113]. A second study analyzed the effects of prenatal exposure to a mixture of seven different anti-androgens (phthalates and pesticides) with differing mechanisms of action (i.e. androgen receptor antagonist or inhibition of androgen synthesis) [46]. All

Table 5
Features of the human population that can influence risks from exposure to environmental contaminants.

Feature	Definition	Example	Implications
Chemical background	Concurrent or preexisting body burdens of environmental chemicals	Over 90% of the U.S. population has measurable levels of plasticizers, pesticides, flame retardants and perflourinated chemicals	Exposure to an individual chemical in addition to other exposures can increase risk of subsequent disease
Biological background	Health status, as influenced by age, pre-existing disease, genetics, and other intrinsic biological factors	About 10% of adults are deficient in thyroid hormone, low thyroid hormone during pregnancy can cause neuro-development harm in children	Chemical exposure has greater effect on people with pre-existing disease, genetic predisposition, etc.
Population variability and defining normal	An individual may have smaller range of normal physiological function than would be observed over the whole population	Individual variability in normal thyroid hormone levels is smaller than the range of normal in the population	Chemical exposures which perturb physiological systems within the "normal" population range may still have effects at the individual level
Small individual effects versus population effects	Chemical exposure may produce a small increase in risk or effect at the level of the individual but result in large shifts in effect at the population level	Observed prenatal phthalate levels slightly decreases the anogenital distance in male babies	US population exposure to phthalates is ubiquitous, so small changes can shift the population distribution of the measurement, resulting in increased effects for a segment of the population
Periods of susceptibility	Exposure during vulnerable periods of development can pose a risk of irreversible effects, both in the short and long term, and diminished capacity for recovery	Proper male reproductive tissue development is dependent on a transient peak in testosterone during fetal development and androgenic disturbances during this period can have unique and permanent affects.	Exposures during susceptible periods can increase risk of permanent adverse effects that may manifest early or not until later in life

Adapted from Woodruff and Janssen [134].

combinations produced cumulative, dose additive outcomes in the androgen-dependent tissues. Swan found that a summary measure of phthalates, integrating exposure to five phthalate metabolites associated with ano-genital distance, was more predictive of the ano-genital distance than the individual metabolite concentrations [114].

2.3.2.5. Conclusion regarding anti-androgen data. The prenatal exposure of males to anti-androgenic chemicals illustrates how findings from animal and human studies together enhance our understanding of effects: (1) animal studies identified critical periods of development sensitive to exposures; (2) animal and human studies propose that perturbations early in the pathway of male reproductive development result in a wide array of permanent and irreversible adverse outcomes; and (3) animal studies show that exposure to different chemicals that affect male reproductive development can have a cumulative effect through different mechanisms of action.

3. Conclusions

The complexity of human disease requires that we draw broadly upon available information to understand the potential impacts of EDCs. Our knowledge of the EDC/disease relationship is strengthened and data gaps reduced when we integrate findings from animal and human studies. Factors that can influence risk, including chemical background exposures, disease status and susceptibility can put a portion of the population at risk from small, incremental exposures, as illustrated in Table 5. Thus, future research efforts should continue to work across disciplines to improve the utility of new research and to contribute to the efforts to understand, and prevent, exposures to harmful chemicals.

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