REPRODUCTIVE TOXICOLOGY

Endocrine disruption and reproductive disorders: impacts on sexually dimorphic neuroendocrine pathways

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Abstract

We are all living with hundreds of anthropogenic chemicals in our bodies every day, a situation that threatens the reproductive health of present and future generations. This review focuses on endocrine-disrupting compounds (EDCs), both naturally occurring and man-made, and summarizes how they interfere with the neuroendocrine system to adversely impact pregnancy outcomes, semen quality, age at puberty, and other aspects of human reproductive health. While obvious malformations of the genitals and other reproductive organs are a clear sign of adverse reproductive health outcomes and injury to brain sexual differentiation, the hypothalamic-pituitary-gonadal (HPG) axis can be much more difficult to discern, particularly in humans. It is well-established that, over the course of development, gonadal hormones shape the vertebrate brain such that sex-specific reproductive physiology and behaviors emerge. Decades of work in neuroendocrinology have elucidated many of the discrete and often very short developmental windows across pre- and postnatal development in which this occurs. This has allowed toxicologists to probe how EDC exposures in these critical windows can permanently alter the structure and function of the HPG axis. This review includes a discussion of key EDC principles including how latency between exposure and the emergence of consequential health effects can be long, along with a summary of the most common and less well-understood EDC modes of action. Extensive examples of how EDCs are impacting human reproductive health, and evidence that they have the potential for multi-generational physiological and behavioral effects are also provided.

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Introduction: reproductive disorders and chemical exposures

Human reproductive health is in trouble. The need for assisted reproductive technology, rates of reproductive cancers, malformations, and similar adversities continue to rise (Swan & Colino 2021). All bodies are also continuously polluted with hundreds if not thousands of chemicals coursing through our tissues and cells every day, including the human placenta and the unborn (Ragusa et al. 2021, Wang et al. 2021). Are these phenomena related? Extensive evidence from across the globe suggests that they are. Although it can be difficult to explicitly link specific chemical exposures to specific reproductive outcomes in humans, the warning signs that show reproductive health is declining as chemical contamination of our environment and ourselves increases, particularly in the last 20–30 years, are irrefutable and concerning. This chapter summarizes this evidence and explores the potential mechanisms by which this occurs with a specific focus on the development and function of the reproductive neuroendocrine system.

Adverse reproductive trends potentially linked to chemical exposures are numerous. One of the most highly publicized is reduced sperm counts in men, which have declined by half in the past 40–50 years (Carlsen et al. 1992, Swan et al. 2000, Geoffroy-Siraudin et al. 2012, Mínguez-Alarcón et al. 2018, Sengupta et al. 2018). Among young Swiss men, only 38% have sperm concentration, motility, and morphology values that meet the WHO semen reference criteria (Rahban et al. 2019). A phenomenon first thought confined to Western populations, the multi-decadal decline in sperm count and quality is now also reported in Africa (Sengupta et al. 2017). Incidence of testicular germ cell cancer, cryptorchidism and hypospadias are rising (Toppari et al. 2010, Manfo et al. 2014), particularly in Nordic countries and other Caucasian populations, signifying a multifaceted decline in male reproductive health.
There are also hints that couple fecundity is declining, with an estimated 10–15% of couples experiencing conception difficulties, but it is extraordinarily difficult to identify its causal factors (Snijder et al. 2012, Buck Louis 2014). Although birth rates in the United States for women aged 20–34 years are falling, elective postponement is a significant driver of that trend, making impacts of chemical exposures difficult to assess. The industrial chemical TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) has dose-dependently been linked to causing longer time to pregnancy in women living in the heavily polluted community of Seveso, Italy (Eskenazi et al. 2010), and there is some evidence that other common pollutants may have similar effects (Wesselink et al. 2018, Kim et al. 2019). Adverse pregnancy outcomes are also increasing, including preterm birth, which, in the United States, has been rising for years and exceeded 10% in 2018 (Martin et al. 2019). Disgracefully, it is among the highest in the developed world, disproportionately impacts people of color, and has been linked, at least in part, to air, agricultural and industrial pollution common in low-income communities (Stieb et al. 2012, Burris & Hacker 2017, Ling et al. 2018).

Female reproductive development, aging, and health is also suffering. According to the American Cancer Society (https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html), in the US women, breast cancer rates have risen to one in eight, and it is now the second most common cancer among the US women. A 2000 study of nearly 45,000 pairs of twins from Sweden, Denmark, and Finland concluded that the environment, not genetics, played a principal role in sporadic cancers of the breast, female reproductive system, and prostate (Lichtenstein et al. 2000). Exposure to dichloro-diphenyl-trichloroethane (DDT), bisphenol A (BPA), and other pollutants have long been suspected to be contributory to rising rates of breast cancer (Davis et al. 1993, Cohn et al. 2015, Rodgers et al. 2018), and there is growing interest in understanding how combined chemical exposures might heighten breast cancer risk (Calaf et al. 2020). It is now irrefutable that aspects of female puberty are advancing (Herman-Giddens 2006, Bourguignon et al. 2016, Boeyer et al. 2018, Fudvoye et al. 2019). In the United States, the median age of breast development and sexual precocity has steadily advanced, especially among minority populations (Herman-Giddens et al. 1997, Partsch & Sippell 2001), a phenomenon that has negative long-term effects on sexual health (Ibitoye et al. 2017). Similar trends have been noted in Europe and among children adopted from developing countries by Western parents (Proos et al. 1991, Parent et al. 2003, Aksglaede et al. 2009). It is less clear if puberty is also advancing in boys (Ohlsson et al. 2019, Reinehr & Roth 2019).

Undoubtedly, the causal factors underlying these and other adverse reproductive health trends for men, women, and couples are complex and multifaceted, but the rapidity of their increased prevalence unequivocally signifies that the primary drivers are environmental. Of these, because the reproductive system is so heavily dependent on steroid and other hormones to develop and function normally throughout life, of greatest concern are chemicals that have the capacity to act on or disrupt endocrine action, a group collectively called endocrine-disrupting compounds (EDCs) (Gore et al. 2015). It was recently estimated that the economic costs of male reproductive disorders and other diseases in men associated with EDC exposure exceed €15 billion annually in the European Union (Hauser et al. 2015). Phthalates and flame retardants were identified as particularly problematic. Similar work has estimated that the burden of these and other EDC-related diseases including intellectual disability, obesity, and neurodevelopmental disorders cost the European Union upwards of €160 billion annually (Bellanger et al. 2015, Trasande et al. 2015). There is also emerging concern that assisted reproductive efforts are compromised in women with higher body burdens of EDCs including phthalates and bisphenols (Al-Saleh et al. 2019, Jin et al. 2019, Mínguez-Alarcón et al. 2019, Shen et al. 2020). Thus, EDCs are likely contributing to a wide range of reproductive maladies in both sexes, along with the extensive emotional, physical and monetary health care costs related to them. When, to how many chemicals, and at what dose levels individuals have been exposed matters in terms of long-term reproductive health risks. Unfortunately, since the 1940s, our collective exposures have only steadily increased with no sign of abating.

Our polluted selves

Chemicals are the words of complex conversations in nature. All living things evolved in a stew of exogenous chemicals, many of which have endocrine action and/or toxicity in extant vertebrates. For example, plants and animals generate potent neurotoxins, including venoms and poisons, for both predatory and defensive purposes. CYP enzymes, required for steroid hormone synthesis and to metabolize toxins, appear to have evolved as a defense against botanical poisons and then repurposed over evolutionary history (Gonzalez & Nebert 1990). Even GABA, the primary inhibitory neurotransmitter of the brain, is an ancient molecule and has a role in plant communication, particularly when the plant is stressed (Ramesh et al. 2015). Plants produce numerous steroids and sterols, most notably the brassinosteroids and phytoestrogens, for a variety of purposes. Not surprisingly, some are hormonally active in vertebrates, including humans (Bishop & Koncz 2002). Notably, some species, particularly those living in perilous environments, evolved mechanisms to leverage endocrine active plant-produced compounds as potent signals of environmental quality and, therefore, optimal
times to invest in reproduction. In that sense, ‘endocrine disruption’ could enhance fitness in hard times (e.g. by suppressing ovulation) or (by enhancing puberty, fertility, or similar) in abundant times. Humans also took advantage of the biological activity of botanicals in traditional and ancient medicine for a variety of purposes including manipulation of reproductive health including assisted labor and elective miscarriage (Kamatenesi-Mugisha & Oryem-Origa 2007, Yazbek et al. 2016, Mbuni et al. 2020). This chemical exchange long conferred evolutionary advantage and heightened fitness. A preliminary form of ‘endocrine disruption’, in some sense, this evolved interplay also signifies how vulnerable our bodies can be to environmental signals including exposure to exogenous chemicals.

Unfortunately, chemicals of our own making are leveraging those evolved relationships to compromise our reproductive health. We blithely eat, breathe, and absorb thousands of chemicals all day, every day, with little to no awareness of their existence, let alone knowledge about their biological activity or potential toxicity. There is no way to know how many chemicals humans have invented or are in commercial use, but there are over 167 million in the CAS Registry, a database maintained by the American Chemical Society, and close to 90,000 on the Environmental Protection Agency’s (EPA) inventory of substances regulated under the Toxic Substances Control Act (TSCA). The vast majority of chemicals in use commercially have not undergone any toxicity testing of any kind. They better our lives in myriad ways and have a wide range of applications including use as plasticizers, surfactants, disinfectants, food preservatives, cleaners, degreasers, fire retardants, solvents, and fragrances. Some have the capacity to interfere with the development and function of the endocrine system and, consequently, reproductive function.

Humans are now born pre-polluted with hundreds to thousands of anthropogenic chemicals (Wang et al. 2016). A landmark study, conducted by the Environmental Working Group in 2005, identified 287 industrial chemicals in umbilical cord blood. A follow-up 2009 study focusing on infants of color confirmed the ubiquity of prenatal pollution. Subsequent studies by multiple groups have repeatedly found harmful chemicals including pesticides and biocides, flame retardants, and per- and polyfluoroalkyl substances (PFAS) in maternal serum and cord blood, with levels sometimes higher in fetal than maternal blood (Wang et al. 2016, van de Bor 2019, Wang et al. 2021). The Centers for Disease Control’s (CDC) National Biomonitoring Program (accessible at: http://www.cdc.gov/nchs/nhanes/index.htm) regularly assesses more than 300 environmental chemicals in Americans and by 2009 indicated universal exposure to many. Significantly, nearly all pregnant women in the United States have at least 43 chemicals with known toxicity in their bodies including polybrominated diphenyl ethers (PBDEs) and other brominated flame retardants, some polychlorinated biphenyls (PCBs) and PFAS, organochlorine pesticides including DDT and its metabolites, BPA, perchlorate, and a variety of phthalates (Woodruff et al. 2011). All 43 have well-documented experimental evidence of toxicity or endocrine disruption and have repeatedly been linked to human health consequences including adverse birth outcomes, childhood morbidity, reproductive cancer risk, and reproductive abnormalities (Wang et al. 2016). Even though some have been phased out of use (e.g. PCBs, DDT, PBDEs), they remain in all of us because their use was so widespread, and they are environmentally persistent. The rapidly expanding catalog of PFAS will also be with us for years and centuries to come. Thus, our ‘exposome’, which is the totality of our bodily exposures, remains poorly defined and easily contains thousands of chemicals (Wishart et al. 2015, Vermeulen et al. 2020).

The earliest evidence that EDCs could adversely impact reproduction largely came from wildlife studies in the 1970s and 1980s including seminal work by Charles Broley, Theo Colborn and Louis Guillette Jr., who linked exposure to DDT, PCBs, and other persistent organic pollutants to reduced fertility in birds, genital abnormalities in alligators, and feminization of numerous species of fish (Leatherland 1992, Guillette et al. 1994, 1995, Beans 1997, Semenza et al. 1997). Around the same time, the clearest evidence that endocrine disruption is plausible in humans emerged from the tragic and misguided use of the potent synthetic estrogen diethylstilbestrol (DES) in pregnancy. Initially prescribed to prevent miscarriage (for which it was ineffective (Reed & Fenton 2013)), it was ultimately dispensed to upwards of 6 million pregnant women in the United States to enhance fetal weight gain (Smith 1948, Karnaky 1953, Kuchera 1971, Palmlund 1996). It was also used as a growth promoter in chicken, cattle, and other livestock, and even used as an ingredient in shampoos, soaps and other personal care products. Human use was suspended in 1971 when prenatal exposure was linked to an extremely rare type of cervicovaginal clear-cell adenocarcinoma (CCAC) in young women (Herbst et al. 1970, 1971). Other uses were phased out years later. In women, prenatal DES exposure has subsequently been linked to vaginal dysplasia, vaginal and cervical adenosis, abnormalities of the cervix, vagina, and uterus, reduced fertility, and greater risk of ectopic pregnancy, late spontaneous abortions, and premature delivery (Reed & Fenton 2013). DES sons have elevated rates of urogenital malformations, undescended testes, testicular cancer, and poor sperm quality (Gill et al. 1976, Stenchever et al. 1981, Wilcox et al. 1995, Palmer et al. 2005).

These and other examples were detailed in the seminal 1996 book Our Stolen Future, Are we Threatening Our Fertility, Intelligence and Survival? – A Scientific Detective
Endocrine disruption defined

There are many working definitions of what constitutes an EDC, some of which are presented in Table 1. The Endocrine Society defines them simply as: chemicals that mimic, block, or interfere with hormones in the body’s endocrine system (Diamanti-Kandarakis et al. 2009, Gore et al. 2015). Others require evidence of an adverse effect on a whole animal. Because our chemical regulatory framework was built decades ago to detect overt poisons and chemicals that cause lethality or cancer, it has struggled to identify a common working definition. EDCs challenge keystone principles of toxicology, including our understanding of what constitutes an ‘adverse’ effect and the long-held axiom ‘the dose makes the poison’. EDCs are not poisons per se (most are not lethal, even at high doses), but rather something else entirely (Schug et al. 2016, Demeneix et al. 2020). The lack of consensus on how EDCs should be defined (Zoeller et al. 2014) has hindered efforts to identify a common list of EDCs. In 2018, the Danish Centre on Endocrine Disrupters published a list (http://cend.dk/files/DK_ED-list-final_2018.pdf) identifying nine chemicals that meet the WHO’s definition of an EDC (Table 2) and four others suspected of meeting it (deltamethrin, 2-(4-tert-butylbenzyl)-propionaldehyde, bifenthrin, hexachlorophene). To date, the USA EPA has failed to identify any, while lists published by other governmental and non-governmental groups contain anywhere from 26 to over 1000 (summarized in a 2020 UNEP report: https://wedocs.unep.org/bitstream/handle/20.500.11822/33807/ARIC pdf?sequence=1&isAllowed=y). Some of the most well-studied putative EDCs linked to adverse reproductive outcomes in animals and humans are listed in Supplementary Table 1 (see section on supplementary materials given at the end of this article). Identified EDCs include numerous pesticides, bisphenols and phthalates, which have multiple applications but are widely used in plastics, and parabens which are often used as preservatives in personal care products. Most identified EDCs that impair reproductive health are

Table 1 EDC definitions currently in use globally.

<table>
<thead>
<tr>
<th>Agency</th>
<th>Year</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>US Environmental Protection Agency (EPA)</td>
<td>1996</td>
<td>An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, and the regulation of developmental processes.</td>
<td>Kavlock and Ankley (1996), Kavlock et al. (1996)</td>
</tr>
<tr>
<td>European Commission Workshop on the Impact of Endocrine Disrupters on Human Health and the Environment</td>
<td>1997</td>
<td>1. An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism or its progeny, secondary to changes in endocrine function. 2. A potential endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism.</td>
<td>European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife 1996</td>
</tr>
<tr>
<td>US EPA Endocrine Disruptor Screening and Testing Advisory Committee</td>
<td>1998</td>
<td>An exogenous substance that changes endocrine function and causes adverse effects at the level of the organism, its progeny, and/or (sub)populations of organisms based on scientific principles, data, weight-of-evidence, and the precautionary principle</td>
<td>EDSTAC 1998</td>
</tr>
<tr>
<td>The World Health Organization (WHO) and the Inter-Organization Programme for the Sound Management of Chemicals (IOMC)</td>
<td>2002</td>
<td>An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations. A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.</td>
<td>WHO/PCS/EDC/0.2.2 (WHO 20002)</td>
</tr>
<tr>
<td>The Endocrine Society</td>
<td>2012</td>
<td>An exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action.</td>
<td>Zoeller et al. (2012)</td>
</tr>
</tbody>
</table>
Endocrine disruption and reproduction

Table 2  A summary of the nine chemicals identified as EDCs by the Danish Center on Endocrine Disruptors in 2018.

<table>
<thead>
<tr>
<th>EDC</th>
<th>Source</th>
<th>Adverse effects</th>
<th>Mode of action</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol AF</td>
<td>Polycarbonate plastics, epoxy resins, food containers, dental fillings, medical devices, eyeglass lenses, thermal paper receipts, electronics</td>
<td>Decreased fertility</td>
<td>Estrogenic</td>
<td>Catenza et al. (2021)</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>Pyrethroid ester insecticide</td>
<td>Malformations of male reproductive organs including the testis</td>
<td>Androgen receptor antagonist</td>
<td>Hodgson and Rose (2007), Ismail and Mohamed (2012)</td>
</tr>
<tr>
<td>Di-n-pentylphthalate (DnPP)</td>
<td>Additive in polyvinylchloride (PVC) and other plastics. No longer in use.</td>
<td>Decreased AGD; increased nipple retention in male rats; decreased sperm count; malformations of male reproductive organs</td>
<td>Anti-estrogenic</td>
<td>Gangolli (1982), Hannas et al. (2011)</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>Organophosphate insecticide</td>
<td>Decreased AGD; increased nipple retention in male rats; malformations of the testis sperm, and male reproductive organs</td>
<td>Anti-androgenic</td>
<td>Curtis (2001)</td>
</tr>
<tr>
<td>Isobutyl paraben</td>
<td>Preservatives in personal care products and pharmaceuticals; food preservatives</td>
<td>Decreased sperm count and motility; altered sexually dimorphic behavior</td>
<td>Estrogenic</td>
<td>Kawaguchi et al. (2010), Gonzalez et al. (2018)</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>Imidazol fungicide; not authorized for use in the United States.</td>
<td>Skewed sex ratios in fish and amphibians; disrupted sex determination in fish and amphibians</td>
<td>Aromatase inhibitor; anti-androgenic; anti-estrogenic</td>
<td>Vinggaard et al. (2006)</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>Phenolic acid; aspirin; personal care products</td>
<td>Reduced spermatogenesis</td>
<td>Anti-androgenic</td>
<td>Kristensen et al. (2016)</td>
</tr>
<tr>
<td>Triclophan (TCC)</td>
<td>Antibacterial; soaps, lotions and other personal care products; largely phased out of use</td>
<td>Increased weight of male accessory sex organs</td>
<td>Androgenic</td>
<td>Rochester et al. (2017)</td>
</tr>
<tr>
<td>Tris(methylphenyl) phosphate</td>
<td>Organophosphate used as a flame retardant and in lacquers, varnishes and as a plasticizer</td>
<td>Reduced fertility/fecundity in adult males</td>
<td>Disrupted steroidogenesis; elevates circulating estrogen levels</td>
<td>Reers et al. (2016)</td>
</tr>
</tbody>
</table>

Estrogenic, anti-androgenic, or, particularly in the case of the phthalates, decrease gonadal steroid synthesis. Compounds produced in nature rather than by humans, such as the phytoestrogens, behave similarly and thus can also be considered EDCs.

Identifying putative EDCs using our current regulatory framework is challenging because of outdated methodologies and other practical limitations (Solecki et al. 2017). Historically, assessments made for regulatory and public health decision-making regarding what is ‘dangerous’ to humans have focused on gross malformations and birth defects such as tumor formation, malformed organs or limbs, severe weight loss, gross motor deficits, fetal loss, and death (Vogel 2013). As the DES story illuminated, EDCs challenge this method of identifying reproductive toxicants because perturbation of the endocrine system may not necessarily lead to overtly obvious teratogenicity post-exposure but rather can set the body on a path to long-term morbidity. This is particularly likely if exposure occurs during developmental periods when hormones are actively shaping neuroendocrine and other reproductive systems.

A powerfully potent estrogen receptor agonist developed as a pharmaceutical, and there are no anthropogenic EDCs that pose a health risk anywhere near as extreme as DES. Nonetheless, most of the reproductive outcomes of prenatal DES exposure are similar to those elicited in many animal models by early-life exposure to some of the most potent phytoestrogens including coumesterol and genistein (Patisaul & Jefferson 2010, Patisaul 2017a); a literature dating back to the 1940s (Bennetts et al. 1946). Additionally, nearly all the human DES outcomes were predicted by or reproduced in animal models (McLachlan et al. 1982, Newbold & McLachlan 1982, Newbold 2008, McLachlan 2016). As such, this work rapidly established a set of endpoints by which reproductive endocrine disruption could be identified, plus a basic mechanistic understanding of how adverse outcomes arise. Moreover, complementary, fundamental work in neuroendocrinology identified more precisely the critical windows in which specific aspects of the reproductive neuroendocrine system differentiate and undergo sexual differentiation (Simerly 2002, Wallen 2009, McCarthy 2016, 2020). Thus, by the close of the
20th century, an experimental framework for testing a broader set of compounds for endocrine disrupting activity across a range of reproductive endpoints was firmly established as well as approaches to establish causality between exposure and adverse reproductive outcomes, and probe mechanisms of action. While EDC scientists have embraced these methodologies and developed new ones as -omics tools advanced and concepts like epigenetic inheritance matured, regulatory testing has largely failed to evolve in either their testing strategies or incorporation of new data streams for the purpose of human risk assessment (Vandenberg et al. 2016, 2019, Solecki et al. 2017, Tweedale 2017, Fritsche et al. 2018). Thus, controversy as to how to precisely define what constitutes an EDC unfortunately remains.

Mechanisms of reproductive endocrine disruption

When the term 'EDC' was first coined in the early 1990s, most chemical examples were estrogenic and thought to act via agonism or antagonism of canonical steroid receptor signaling (Hotchkiss et al. 2008, McLachlan 2016). Thanks to convergent work in endocrinology, toxicology, and behavioral neuroendocrinology, significant progress has been made identifying potential mechanisms of reproductive endocrine disruption beyond classic steroid hormone receptor agonism/antagonism. These 'key characteristics' (Fig. 1) include changes in steroid metabolism and biosynthesis, receptor degradation and expression level changes, DNA methylation and other epigenetic modifications, interference with signal transduction in hormone-responsive cells and disruption of rapid hormone signaling via non-canonical pathways (Tabb & Blumberg 2006, Schug et al. 2015, La Merrill et al. 2020).

While it is beyond the scope of this review to address them all in detail, it is important to note that for each key characteristic, there can be multiple mechanisms of disruption. For example, the earliest identified EDCs were estrogen disruptors. These EDCs were presumed to act by interfering with direct genomic signaling, where an estrogen (17β-estradiol, estrone, or estriol) binds to either major form of the estrogen receptor (ERα, ERβ), and the complex dimerizes and translocates to the nucleus inducing transcriptional changes in estrogen-responsive genes via estrogen response elements (EREs). To this day, most in vitro assays that screen for estrogen-disrupting EDCs only test this mechanism of action. It is now known, however, that endogenous estrogens can act via indirect genomic signaling, where another transcription factor mediates DNA binding at a non-ERE site. Additionally, membrane-bound receptors, including a membrane form of ERα, can induce cytoplasmic events such as modulation of membrane-based ion channels, second-messenger cascades, or transcription factors (Fig. 2). Notably, the G protein-coupled estrogen receptor (GPER, also known as GPER1 or GPR30), first discovered in 1996, is expressed in the cell membrane as well as the endoplasmic reticulum and plays an important role in many of the rapid non-genomic estrogen actions including heightened production of cyclic AMP, promotion of intracellular calcium mobilization, and synthesis of phosphatidylinositol 3,4,5-trisphosphate in the nucleus (Filardo & Thomas 2012, Qie et al. 2021). EDCs known to bind or interfere with GPER include BPA and other bisphenols, some pesticides including DDT.
and its metabolites, many flame retardants including PBDEs and some organophosphate esters (OPEs), phthalates, and some endocrine active metals such as cadmium (Qie et al. 2021). Additionally, ER-independent and estrogen-independent actions are also known to exist and could be impacted by EDCs (Fig. 2). This is why some tissues which primarily express only one form of the estrogen receptor (e.g. ERβ in granulosa cells, ERα in thecal cells, and membrane ERs in some brain regions such as the nucleus accumbens) respond differently to endogenous estrogens and, consequently, vary in their vulnerability and response to estrogen-disrupting EDCs (Fucic et al. 2012, Hewitt & Korach 2018). Emerging work is also demonstrating that organs other than the gonads can make their own estrogens, most significantly the brain (Barakat et al. 2016). Similar receptor signaling complexities exist for androgens (Mittelman-Smith et al. 2017) and progestins as well (Piette 2020), with some ligands binding other receptors including glucocorticoid receptors (Mittelman-Smith et al. 2017).

EDCs are now also recognized as being able to induce epigenetic changes, and this is particularly alarming because it provides a mechanistic path to transgenerational effects. When a pregnant woman is exposed to a chemical, she, her unborn child, and the germ cells in that unborn child are all simultaneously exposed. Thus, exposure is to three generations at once and considered multi-generational. The fourth generation, (called F4) or the great-grandchild, is the first to be born not directly exposed. Effects in this F4 generation are considered transgenerational. There is growing evidence that a multitude of EDCs has transgenerational effects on reproductive endpoints including age at puberty, fertility and fecundity, reproductive behavior, and parental behavior (Rissman & Adli 2014, Ho et al. 2017, Viluksela & Pohjanvirta 2021).
2019). These can occur via a variety of epigenetic mechanisms, most of which are only beginning to be fully understood (Walker & Gore 2011) but include DNA methylation, histone modifications, and long and micro RNAs among others (Perera et al. 2020). Although the evidence remains sparse and inconsistent, particularly in humans (Van Cauwenbergh et al. 2020), there is pressing interest in the possibility of transgenerational outcomes, particularly in the face of other life challenges, such as prenatal stress or poor nutrition, which could augment adverse generational effects (Sobolewski et al. 2020). As such, epigenetic effects and, consequently, the potential for transgenerational inheritance are some of the most intense areas of reproductive EDC research.

The reproductive neuroendocrine system

The neuroendocrine system is essentially the master control system of the body and the primary system responsive to environmental signals. The nervous system component mediates the most immediate and rapid effects, and the endocrine level acts to maintain, modulate, and prolong the response. For example, when frightened, the sympathetic nervous system drives the immediate response (e.g. increased heart rate, sweating and goosebumps) while the endocrine system acts to maintain a stress response (via glucocorticoids and other hormones). The neuroendocrine system, especially the reproductive neuroendocrine system, is exquisitely sensitive to steroids and other hormones throughout life. Itself an endocrine organ, the hypothalamus is the apical coordinator of the neuroendocrine system. This diencephalic, heterogeneous, and sexually dimorphic structure communicates with the rest of the brain, the pituitary and, ultimately, all the endocrine glands including the gonads. The placenta also shapes and controls many neuroendocrine functions in the developing fetus, including the organization of the fetal brain, across pregnancy, beginning well before the hypothalamus is even formed (Behura et al. 2019). Thus, it is an ephemeral but critical piece of the neuroendocrine system.

The vertebrate reproductive system is coordinated by the hypothalamic-pituitary-gonadal (HPG) axis, which comprises a complex network of neuronal and endocrine signaling pathways that ultimately centers on the precise control of steroid hormone secretion by gonadotropins (McCarthy et al. 2009, Schulz et al. 2009, Kapra & Huhtaniemi 2018). HPG steroid hormone action can be organizational or activational depending on the developmental stage (McCarthy et al. 2009, Schulz et al. 2009), and over the pubertal transition, there is a little bit of both (Schulz & Sisk 2016). The neural components of the HPG axis span multiple hypothalamic and other brain nuclei, most importantly regions housing discrete populations of kisspeptin-secreting neurons that regulate gonadotropin-releasing hormone (GnRH) secretion. In humans, a preoptic population coordinates ovulation and steroid positive feedback in females, while the more caudal arcuate (also called the infundibular nucleus) population regulates pubertal onset and steroid negative feedback in both sexes (Livadas & Chrousos 2016, Barroso et al. 2019, Garcia et al. 2019). Females have more preoptic kisspeptin neurons than males and estrogen upregulates kisspeptin expression in this region via ERα in both sexes which then, in turn, triggers the pre-ovulatory surge of GnRH release in females (Beltramo et al. 2014, Herbison 2020). The ARC population of kisspeptin neurons co-synthesize neurokinin B (NKB) and dynorphin and have thus come to be termed ‘KNDy neurons’ (Moore et al. 2018). Estrogens suppress the kisspeptin expression in KNDy neurons via membrane ERα, and neuron numbers are not sexually dimorphic (Micevych et al. 2015). KNDy neurons are also critical for metabolic control and are considered key integrators of reproductive and metabolic signaling pathways (Dudek et al. 2018). The network of regulatory inputs from other neural and glial cells in the brain projecting to these kisspeptin populations or to GnRH neurons themselves is diverse, and in many cases, highly sexually dimorphic. Described in detail elsewhere, these pathways are differentiated largely by endogenous gonadal hormones through a series of well-defined, and sometimes very short, gestational, pre- and perinatal critical periods (Simerly 2002, Clarkson et al. 2014, Clarkson & Herbison 2016, McCarthy et al. 2018) spanning gestation through pre-puberty (Simerly 2002, Schulz et al. 2009).

Endocrine disruption of HPG axis sexual differentiation

In humans and other mammals, the fetal testis is steroidogenically active and the secreted testosterone and its metabolites are required to masculinize the brain, genitalia and reproductive tract. Androgens, most significantly dihydrotestosterone, via their action on androgen receptors, are essential for masculinizing the male reproductive organs. By contrast, in rats, mice and some other non-primate species, the masculinizing effect of perinatal androgens in the brain is predominantly conferred by estrogens, derived locally via aromatization, and precisely expressed estrogen receptors (McCarthy 2008, Cao & Patisaul 2011, 2013). Thus, in rodents, estrogen is the 'masculinizing' gonadal hormone of the perinatal brain. In humans, although aromatization occurs, androgen receptors play a far more direct role in brain masculinization, a process that continues after birth (Gooren 2006, Alexander 2014, Savic et al. 2017, Puts & Motta-Mena 2018). This is critically important to keep in mind when interpreting and translating effects on rodent hypothalamic sexual differentiation to humans. Estrogenic action in the perinatal brain will be masculinizing in rodents but not
humans or other primates. It is also important to note that the neuroendocrinology of sexual orientation and gender identity remain largely unknown (Roselli 2018).

In many species, the sex-specific organization of GnRH function and feedback can be hormonally manipulated and induce long-term functional consequences. During the perinatal critical period, gonadal steroid hormone exposure can masculinize the female rodent GnRH axis, while neonatal castration can effectively prevent defeminization of the male GnRH axis (Baum 1979, Simerly 2002, Bakker & Baum 2008). Thus, in males castrated as neonates, the potential for estrogen to evoke a GnRH surge is preserved while, conversely, in females neonatally exposed to estrogens, this capacity is diminished or lost. While this critical window is perinatal in rats and mice, it is thought to be entirely prenatal in humans.

Because steroid hormones are essential for the proper organization and sexual differentiation of the perinatal reproductive neuroendocrine system, this process has proven particularly vulnerable to endocrine disruption, especially in males (Kern et al. 2017, Graceli et al. 2020). For example, in rats, fetal exposure to some phthalates particularly di-(2-ethylhexyl phthalate (DEHP), genistein, coumestrol and other phytoestrogens, PCBs, as well as BPA and likely other bisphenols perturb programming in multiple hypothalamic and related nuclei including the ARC, anteroventral periventricular nucleus (AVPV) and medial preoptic nucleus (MPN) (Dickerson et al. 2011, Jefferson et al. 2012, Bell 2014, Gao et al. 2018, Patisaual 2020). These disruptions include changes in nuclear volume, expression of steroid hormone and other receptors, the density of projections between brain nuclei, and density of dopaminergic neurons in regions critical for reproductive function (Frye et al. 2012, Gao et al. 2018, Neubert da Silva et al. 2019, Patisaual 2020). EDCs including genistein, BPA and PCBs can be disruptive to the organization and to the function of the GnRH-kisspeptin system (Tena-Sempere 2010, Patisaual 2013). Neonatal genistein, for example, can masculinize the female rat GnRH axis such that capacity for steroid positive feedback is compromised (Bateman & Patisaual 2008). Rat females exposed for only the first 3 days of life have fewer kisspeptin immunopositive fibers in both the AVPV and ARC across the pubertal transition, an effect accompanied by evidence of disrupted ovarian maturation (Losu et al. 2011). Genistein and BPA exposure over only the first 48 h after birth is sufficient to alter other aspects of AVPV sexual differentiation including the number of dopaminergic neurons (Patisaual et al. 2006). This finding is significant because GnRH neurons express dopamine receptors, and dopamine is a potent modulator of pre- and postsynaptic actions on GnRH neurons (Liu & Herbison 2013, Spergel 2019).

Similarly, female mice perinatally exposed to BPA have an early vaginal opening (a marker of rodent puberty), lower circulating levels of LH, and significantly altered numbers of AVPV and ARC kisspeptin neurons (Ruiz-Pino et al. 2019). Perinatal BPA exposure has also been shown to dysregulate the GnRH axis, including kisspeptin neuron numbers, in rats (Bai et al. 2011).

In addition to the HPG axis, some EDCs can disrupt the sexual differentiation of other hypothalamic systems. For example, in mice, prenatal exposure to a mixture of common OPEs used as plasticizers and flame retardants eliminates the sex difference in postnatal Npy (neuropeptide Y) expression in the mediobasal hypothalamus (MBH) and increases the postnatal expression of ERa and other genes related to sexual differentiation, including Pparg (peroxisome proliferator-activated receptor gamma), Tac2 (tachykinin 2), and Pdyn (prodynorphin) in females (Adams et al. 2020). Exposed males were also heavier by the second week of life and had higher levels of Agrp (agouti-related neuropeptide) in the MBH at birth, supporting other studies identifying them as metabolic disruptors (Farhat et al. 2014, Boyle et al. 2019). OPEs have also been found to disrupt HPG function and impair reproduction in fish (Liu et al. 2013, Saunders et al. 2015, Wang et al. 2015). There is also evidence from multiple species including quail and monogamous rodent models that BPA, PCBs and some phthalates alter sexually dimorphic aspects of the oxytocin/vasopressin system and their interface with the mesolimbic dopamine system (Sullivan et al. 2014, Patisaual 2017b, Gore et al. 2019).

In humans, evidence of disrupted hypothalamic sexual differentiation is extremely difficult to detect, but anogenital distance (AGD) has emerged as a potentially useful biomarker of fetal androgen exposure and, consequently, potentially altered the brain defeminization / masculinization (Schwartz et al. 2019). Due to prenatal virilization by androgens, AGD is longer in males than females. Although links to specific brain outcomes are not yet well-defined, shorter male AGD is associated with reduced fertility, sperm quality and circulating androgen levels as well as fewer male-typical play patterns (Thankamony et al. 2016, Priskorn et al. 2019). In addition to shortened AGD, fetal phthalate exposure results in male genital and reproductive organ abnormalities and higher testicular cancer risk in rodent models and humans (Hlinsikova et al. 2020) which is sometimes termed ‘testicular dysgenesis syndrome’ (Sharpe & Skakkebaek 2008, Toppari et al. 2010). Fetal exposure to acetonaphen (Paracetamol or Tylenol), BPA, DDT and its primary metabolites, dioxins, phthalates, some OPEs, and the dicarboximide fungicides such as vinclozolin can also result in shortening AGD and, consequently, are thought to impair HPG masculinization (Schwartz et al. 2019, Adams et al. 2020). Similarly, longer AGD in females is considered an indicator of masculinization, the long-term reproductive consequences of which are not entirely clear. Notably, ectopic fetal activation of the progesterone receptor (PR) can masculinize female
genitalia, as can super-physiological doses of potent estrogens, so there are likely multiple modes of action for disrupted AGD length in females. Some EDCs including BPA, paracetamol and a few phthalates have been shown to shorten female rodent AGD, the functional significance of which is unknown. Thus, the relationship between AGD length and HPG sexual differentiation in females has yet to be established. Significantly, AGD measurements have been added to several OECD test guidelines and other regulatory testing strategies for developmental and reproductive toxicity for detecting endocrine-disrupting effects. Its utility for predicting adverse reproductive outcomes in humans is less well-accepted but gaining traction.

Disruption of HPG maturation and adult function

In experimental animals, it is well-established that the administration of steroid hormones during adolescence can accelerate puberty including the premature onset of GnRH pulsatility (Rasier et al. 2006, Mueller & Heger 2014, Lucaccioni et al. 2020). Disruption of HPG axis maturation and pubertal onset has been observed in rodents following exposure to BPA, phthalates, atrazine, DDT and its metabolites, TCDD, and PCBs (Gore et al. 2015). In most cases, female puberty is advanced but high doses of potent estrogenic compounds can also induce a delay. For example, in female rats, parabens administered during peripuberty delayed puberty and resulted in numerous uterine and ovarian histological changes including decreased corpora lutea, increased incidence of cystic follicles and myometrial hypertrophy (Vo et al. 2010).

A far longer list of EDCs including genistein, coumestrol and other phytoestrogens, DDT, atrazine, BPA and other bisphenols, and some PCBs are known to interfere with the activity of the mature HPG axis and thus disrupt GnRH and LH secretion (Gore et al. 2015, Rattan et al. 2017, Graceli et al. 2020). This can occur via direct action on GnRH neurons or other hypothalamic targets but disruption at the level of the pituitary or gonad is more common. In most cases, normal HPG function resumes once exposure is eliminated. For example, a 2009 meta-analysis concluded that isoflavone intake moderately increases cycle length and suppresses luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in adult women (Hooper et al. 2009). A 2008 clinical case report described three women (aged 35 to 56) experiencing a suite of symptoms related to excessive soy intake (estimated to exceed 40 g per day) including abnormal uterine bleeding, endometrial pathology and dysmenorrhea, all of which resolved when soy intake was discontinued or reduced (Chandrareddy et al. 2008). For couples experiencing fertility challenges, it can be difficult to establish if developmental or current exposures (or both) may have adversely contributed, but assisted reproductive technologies are compromised in women with high blood levels of BPA, phthalates, and other EDCs (Karwacka et al. 2019, Mínguez-Alarcón et al. 2019).

Finally, only limited data exist on the impact of EDCs on reproductive senescence and disorders of the aging reproductive system. Estrogenic EDCs including PCBs and phytoestrogens are associated with a higher risk of uterine fibroids, a condition that impacts up to 80% of women in their lifetime and is highly dependent on estrogen and progesterone (Katz et al. 2016). Higher blood levels of DDT and its metabolites are associated with an early age at menopause (Akkina et al. 2004) while other pesticides have been associated with later age at menopause (Farr et al. 2006). There is also some evidence that TCDD and PCBs can advance female reproductive aging (Rattan et al. 2017) and BPA heightens the risk of prostate cancer (Prins et al. 2014, Gore et al. 2015). Effects of phytoestrogens are mixed with conflicting evidence that they are possibly cancer-inducing or protective, most likely depending on dose, the timing of exposure, and other factors (Patisaul & Jeffersson 2010). Impacts on other aspects of reproductive aging, including premature depletion of ovarian follicle reserves, are also likely but underexplored (Weiss 2007, Grossman 2014, Johansson et al. 2016, Barakat et al. 2017).

The placenta

There continues to be growing recognition that the placenta is not an impenetrable barrier but, rather, may be a particularly vulnerable target for endocrine disruption (Wang et al. 2016, Gingrich et al. 2020). It is now clear that the bulk of EDCs likely reach the developing fetus (Aylward et al. 2014). For example, maternal estrogens are effectively sequestered by α-fetoprotein but most structurally similar compounds only weakly or fail to bind to α-fetoprotein and can, therefore, enter fetal circulation relatively unimpeded (Milligan et al. 1998, Ikuzuki et al. 2002, Vandenberg et al. 2007). Environmental contaminants known to cross the placenta and reach the fetus include BPA and other phenols, phthalates, heavy metals, pesticides, PFAS, and numerous flame retardants (Leazer & Klaassen 2003, D’Aloisio et al. 2013). Some pollutants can accumulate in placental tissue (Esteban-Valasalo et al. 2012, Vizcaíno et al. 2014, Leonetti et al. 2016a,b, Phillips et al. 2016, Baldwin et al. 2017) and appear at higher levels in fetal cord blood than maternal blood (e.g. some metals, brominated flame retardants including TBBPA and many PBDE congeners, and polyaromatic hydrocarbons) (Aylward et al. 2014), demonstrating that fetally derived placental tissues and the fetus itself can experience greater exposures than the mother. In the case of some flame retardants, this placental accumulation appears to differ by sex with placentas associated with the male offspring obtaining higher levels, raising the possibility...
that fetal exposure may differ by sex (Phillips et al. 2016, Rock et al. 2018). The placenta is the site of nutrient exchange between mother and fetus, but it also provides critical support for fetal growth through hormone and neurotransmitter production. Not surprisingly, the placenta has been found to play a unique and critical role in neurodevelopment, and, consequently, dysfunction of the placenta can severely impact neurodevelopment (Myatt 2006, Konkel 2016). There are many examples of situations where stress-induced inflammation can lead to placental dysfunction and altered neurodevelopment (Bronson & Bale 2014, 2016), however, comparatively few studies have tested whether this type of response can occur from exposure to exogenous chemicals (Gingrich et al. 2020, Mao et al. 2020). Thyroid hormone regulation and transfer are also vital for proper neurodevelopment and highly susceptible to EDCs such as PBDEs and PCBs (Demeneix 2014). Therefore, during the gestational window, neurodevelopment can not only be impacted by direct toxicity to the developing brain, through placental transfer, but can also be impacted indirectly through placental disruption.

Other routes of reproductive endocrine disruption

Endocrine disruption does not have to be direct. Because the neuroendocrine pathways that coordinate reproductive physiology intersect with other organ systems and pathways, even indirect environmental insults can have reproductive consequences. For example, chronic stress including prolonged isolation, parental neglect, starvation, or extreme exercise can itself suppress ovulation, induce miscarriage, reduce sperm count, result in preterm birth, and shift the timing of pubertal maturation. Additionally, dysregulation of the stress axis can contribute to other chronic health conditions including metabolic syndrome, impaired immune function, cardiovascular disease, and cognitive decline (Dirven et al. 2017, Joseph & Golden 2017) highlighting the multi-organ system impacts of neuroendocrine disruption. Similarly, the endocrine and immune systems reciprocally influence each other, and hyperinflammation can suppress fertility and reproduction (Belloni et al. 2014). Reproductive maturation and function are also under strong metabolic control and highly sensitive to body condition (Bellefontaine & Elias 2014). In combination with stress, chronic illness and other bodily insults, the severity and breadth of EDC-related health effects can be compounded. For accelerated female puberty, for example, commonly ascribed causal factors such as father absence and obesity have turned out to play less of a singular role than once believed (Li et al. 2017, Sohn 2017, Reinehr & Roth 2019, Sear et al. 2019). However, in combination with other exogenous insults including chemical exposures, the risk of precocious puberty and other reproductive challenges increase. The degree to which EDCs factor into this complex landscape and influence reproductive outcomes in humans remains difficult to quantify with certainty, but experimental evidence strongly implicates that their impact is likely underestimated and growing.

Finally, many EDCs have the potential to elicit their effects through modes of action that impact other organ systems. The endocrine system has been shown to interact with the immune system, for example, by mediating gene transcription of pro-inflammatory cytokines, and mediators of the innate immune system can feedback on the brain and regulate endocrine signaling (Irwin & Cole 2011, Merrheim et al. 2020). PBDEs and PFAS have been associated with inflammation during pregnancy and the postpartum period in women (Zota et al. 2018). Additionally, uterine remodeling, driven by immune cells, is critical for preparing the uterus for pregnancy, a process that may be vulnerable to EDCs (Meyer & Zenclussen 2020). In the brain, microglia play an essential role in the execution of sexually dimorphic hypothalamic development (Lenz & McCarthy 2015). Consequently, during development, their densities can be sexually dimorphic in hypothalamic nuclei (Schwarz et al. 2012) and environmental exposures, including exposure to air pollution or BPA, can sex-specifically alter their numbers or morphology, particularly in combination with other stressors (Bolton et al. 2013, Hanamsagar & Bilbo 2016, Rebuli et al. 2016, Bolton et al. 2017). Interest in the capacity for EDCs to disrupt the microbiome and the brain-gut axis is also increasing (Roman et al. 2019, Kaur et al. 2020).

Take-home messages: key concepts of endocrine disruption in the reproductive system

The DES disaster illustrates three core principles of endocrine disruption. First, latency between exposure and effect can be extremely long, even decades. DES-exposed babies were born healthy by all appearance, with no evidence of the reproductive consequences that would ultimately befall them. Pioneering work by Drs Beach, Young, Goy and others exploring the mechanisms by which fetal hormone exposure alters sex behavior and sex-specific neuroendocrine feedback systems (Gorski 1963, Young et al. 1964, Goy & Resko 1972, Swaab & Hofman 1984, Balthazart et al. 1996, Marler 2005) identified the primary mechanisms underlying this phenomenon in the reproductive system. As such, neuroendocrinologists laid critical groundwork for conceptually understanding how reproductive development and function could be vulnerable to endocrine disruption. This long latency concept is now a cornerstone of the ‘developmental origins of health and disease (DoHAD)’ framework; a concept born from the Barker hypothesis stating that metabolic adaptations made during early-life scarcity could later heighten the
risk of morbidity in an environment of plenty (Barker 2007, Heindel et al. 2015).

The second key principle is that the timing of exposure, perhaps even more than dose, drives the potential and severity of adverse outcomes. In vertebrates, there are critical moments, both for the reproductive organs and the brain, when sensitivity to endogenous hormones and, consequently, EDCs is especially high. Additionally, there are phases in the life trajectory, particularly in prenatal development, where hormones play an organizational role in the formation of reproductive systems. Because interference with those organizational events can result in irreversible injury, they are considered particularly critical windows of EDC vulnerability. For example, the type and severity of disorders common to DES sons and daughters vary substantially depending on the timing of the mother’s first exposure, total dose, and length of exposure (Robboy et al. 1981, 1984, Faber et al. 1990). The vast majority of experimental EDC research on reproduction has used prenatal or early-life exposure paradigms and exposure later in life assessment. By contrast, exposures during puberty, which is an additional, but not as well-characterized period of organizational hormone action, are not as well-studied (Ahmed et al. 2008, Schulz et al. 2009, Sisk 2016, Herting & Sowell 2017). Also underexplored is the possibility that EDC exposure changes the timing of when and for how long critical windows are open. Thyroid hormones have long been known to open the window for filial imprinting in birds and can reopen that window in some circumstances (Yamaguchi et al. 2012). Embryonic exposure to BPA and BPS has been shown to shift the timing of neurogenesis in the zebrafish brain (Kinch et al. 2015). Almost nothing is known about how windows of HPG organization can be shifted in the mammalian brain by EDCs.

The third key principle of endocrine disruption is that the dose-response of many hormones and EDCs appears to be non-linear, and adverse outcomes might be different at low vs high doses (Vandenberg et al. 2012). The best example of this is thyroid hormone because the effects of abnormally high or low levels are completely different. In adults, symptoms of hyperthyroidism include bulging eyes, weight loss, tachycardia, arrhythmias, goiter, heat intolerance, and anxiety or nervousness, while hypothyroidism can result in weight gain, fatigue, cold intolerance, muscle and joint pain, hair loss, dry skin, depression and menstrual irregularity. This paradigm is anathema to the fundamental toxicological axiom that ‘the dose makes the poison’ which posits that effects intensify with dose but do not fundamentally change, nor manifest differently, below a certain threshold. Homeostatic adaptation can also produce non-linear dose responses. Our neuroendocrine system evolved to be responsive to environmental signals, both chemical and perceived, including naturally occurring hormonally active compounds. Consequently, we have adaptive responses to some environmental insults and can make imperceptible adjustments to maintain homeostasis, but only up to a point. For example, the neural components of the HPG axis are constantly responding to numerous external signals simultaneously including day length, hormones, infection, olfactory cues, and glucose levels, among others. Complex feedback loops on the HPG axis maintain physiologically appropriate levels of steroid hormones and rhythmic GnRH pulses. These feedback systems are likely one reason why many EDC dose responses more closely approximate a U-shaped or inverted U-shaped curve (Vandenberg et al. 2012, Lagarde et al. 2015, Zoeller & Vandenberg 2015). U-shaped dose effects might also reflect an integration of two different mechanisms of action, each of which occurs at a different dose range. For example, DDT interferes with estrogen and androgen action at low doses but is a potent neurotoxin at higher levels (Toppari et al. 1996). It should be noted that although some toxicologists have expressed concern that non-linear dose responses are underappreciated, others doubt their existence entirely, and the concept remains highly controversial for some, primarily because it would necessitate changing the way regulatory toxicity testing is conducted (Melnick et al. 2002, Vandenberg et al. 2012, Lagarde et al. 2015, Solecki et al. 2017, Demeneix et al. 2020). Others have also perturbed this concept and labeled it ‘hormesis’, a largely debunked hypothesis which purports that low doses of some toxic compounds can actually be beneficial even if high doses are harmful (Thayer et al. 2006, Kendig et al. 2010).

Concluding thoughts

Human evolution will now occur in a complex chemical landscape of our own making. It is readily evident that exposure to EDCs is already adversely affecting reproductive physiology and behavior in ourselves and our planetary co-occupants and will continue to do so for generations to come. Growing evidence that some EDCs can induce epigenetic reprogramming that may be inherited and impact future generations emphasizes the pressing need to understand how the chemical cocktail inside of us is impacting our reproductive future (Ho et al. 2017). Some of the most obvious and potent compounds such as DDT, PCBs, PBDEs and DES, have been phased out of use and, fortunately, global exposure is consequently declining. For others such as BPA and the phthalates, despite an enormous literature documenting their adverse reproductive effects, regulatory inaction persists. The introduction of new chemicals is rapidly outpacing the capacity to understand their potential health effects or the totality of our exposure (Tweedale 2017). Additionally, even perceived successes are often failures because all too often problematic compounds are replaced by equally problematic cousins, a phenomenon called ‘regrettable
substitution’ (Scherer et al. 2014, Trasande 2017, Blum et al. 2019). Collectively, blood levels of many hormonally active compounds can be several fold higher than endogenous estrogen levels, particularly during pregnancy and neonatal development, and are almost always present in both the environment and in tissues as mixtures (Wang et al. 2016). Yet, regulatory processes overwhelmingly consider their potential harm in isolation, one chemical at a time. Further efforts to understand the mechanisms underlying EDC effects, particularly complex mixtures of ubiquitous compounds with low hormonal potency, are necessary to adequately develop a public health strategy for preventing or combating their reproductive effects (Bopp et al. 2018). That every pregnant woman on the planet is currently carrying a mixture of chemicals during her pregnancy that could affect not only her daughter’s reproductive health but also her granddaughter’s is a major reason why the topic of endocrine disruption must continue to receive global attention from scientists, governments, and the general public.

Supplementary materials
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