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Reproductive and Metabolic Health Following Exposure to Environmental Chemicals: Mechanistic Insights from Mammalian Models

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Abstract

The decline in human reproductive and metabolic health over the past 50 years is associated with exposure to complex mixtures of anthropogenic environmental chemicals (ECs). Real-life EC exposure has varied over time and differs across geographical locations. Health-related issues include declining sperm quality, advanced puberty onset, premature ovarian insufficiency, cancer, obesity, and metabolic syndrome. Prospective animal studies with individual and limited EC mixtures support these observations and provide a means to investigate underlying physiological and molecular mechanisms. The greatest impacts of EC exposure are through programming of the developing embryo and/or fetus, with additional placental effects reported in eutherian mammals. Single-chemical effects and mechanistic studies, including transgenerational epigenetic inheritance, have been undertaken in rodents. Important translational models of human exposure are provided by companion animals, due to a shared environment, and sheep

exposed to anthropogenic chemical mixtures present in pastures treated with sewage sludge (biosolids). Future animal research should prioritize EC mixtures that extend beyond a single developmental stage and/or generation. This would provide a more representative platform to investigate genetic and underlying mechanisms that explain sexually dimorphic and individual effects that could facilitate mitigation strategies.

1. INTRODUCTION

Over the last 50 years, a decline in human reproductive and metabolic health has been seen across the world. Exposure to the complex low-level mixtures of anthropogenic chemicals that are ubiquitous within the modern environment appears to be a contributory factor. The structural similarity of some of these environmental chemicals (ECs) to endogenous hormones allows them to directly activate and/or block endogenous hormone receptors, resulting in the modification/disruption of normal endocrine regulation health (1, 2). Where the structure of an EC resembles a sex hormone, or where the actions of a hormone are sex specific, these direct effects of ECs can be sexually dimorphic. ECs can also impact health indirectly, through pathways that alter reproductive and metabolic processes such as oxidative stress and DNA damage, apoptosis, inflammation, and epigenetic regulation, and again, such effects can be sexually differentiated. Human epidemiological studies have provided extensive evidence on the effects of exposure to certain ECs and some EC mixtures (e.g., cigarette smoking) and the developmental programming of long-term health. However, the nature of everyday EC exposure is complex and ever changing, which poses great challenges in determining exposure risk. To address this, animal models have been used to study the effects of both single and mixed EC exposure on various health outcomes. This review critiques key studies that have used animals to gain insights into mechanisms (including epigenetics) through which ECs can alter metabolic and reproductive health, and how animal models can be used to address unanswered questions relating to effects of exposure to low-level EC mixtures.

2. OVERVIEW OF EC EXPOSURE

The environment contains a plethora of ECs from various sources. These include the so-called persistent organic pollutants (POPs), such as the pesticide dichlorodiphenyltrichloroethane (DDT); polychlorinated biphenyls (PCBs, which were used as heat-transfer fluids and as dielectric and coolant fluids); polyfluoroalkyl substances (PFAS, found in firefighting foams and textiles); polybrominated compounds, e.g., polybrominated diphenyl ethers (PBDEs, often used as flame retardants); dioxins (a by-product of the manufacturing of pesticides and construction materials); and polychlorinated dibenzodioxins (e.g., polychlorinated dibenzo-p-dioxins, generated as a consequence of the incomplete combustion of chlorine-containing substances) (3, 4). Other ECs include organophosphate pesticides (e.g., parathion and malathion); polycyclic aromatic hydrocarbons (PAHs) from combustion of fossil fuels; plasticizers such as bisphenol A (BPA) and phthalate esters, for instance, diethylhexyl phthalate (DEHP) (5); pharmaceuticals (e.g., antibiotics, chemotherapeutics, psychotropics); and natural and synthetic hormones (6–8). Once released, ECs can (*a*) become widely distributed throughout the environment as a result of natural processes, (*b*) persist for exceptionally long periods of time (even after their production and use has been discontinued), (*c*) accumulate in living organisms including humans, (*d*) bioaccumulate to greater concentrations at higher trophic levels, and (*e*) induce toxic effects on both humans and wildlife.

Since the 1950s, more than 140,000 new chemicals have been produced, and the number and quantity of anthropogenic chemicals released into the environment have increased simultaneously.

ECs are readily detectable in air, water, and soil (7), and although their distribution patterns show some geographical variability, typically due to local regional prohibition of their production and use, most countries show contamination from a wide but similar range of industrial/anthropogenic chemicals (8). The exposure of humans and domestic animal and wildlife species to a mixture of ECs is, therefore, inevitable. Despite a global EC monitoring plan initiated to monitor worldwide trends in the concentrations of POPs listed under the Stockholm Convention (2, 9), there is little or no environmental assessment of the singular or, potentially more importantly, additive/cumulative effects of complex EC mixtures that mirror real-life EC exposure. Traditionally, chemical risk assessments are based on the no-observed-adverse-effect level (NOAEL), which is determined by testing the effects of individual chemicals in rodents or cell-based assays. Although NOAELs are invaluable for individual chemical toxicological assessment, they can be orders of magnitude higher than that of human EC exposure. Furthermore, the toxicological profile of a mixture of ECs cannot be determined from NOAELs of each component chemical in the mixture. Studies of the potential risk posed by exposure to mixtures of ECs have used mathematical models based on the individual chemicals within a mixture (10). However, they typically do not account for synergistic or antagonistic interactions between chemicals and the cumulative/additive/synergistic effects of ECs present in mixtures that are below the NOAEL.

3. GLOBAL METABOLIC AND REPRODUCTIVE HEALTH ISSUES

Over the last 50 years, there has been a striking increase in global obesity (9, 11), and by 2025, 20% of the world population is predicted to be obese. In association with this obesity pandemic, there has been a dramatic increase in the incidence of metabolic syndrome (MetS), which is an associated cluster of clinical metabolic symptoms including increased blood pressure, high blood sugar, lower abdominal fat deposition, and abnormal cholesterol or triglyceride levels (12–15). MetS affects an estimated ~35% of adults and 50% of those aged 60 years or older in the United States (16). MetS is a societal health concern, as it predisposes individuals to insulin resistance, hyperglycemia, dyslipidemia, and cardiovascular disease (CVD) and dramatically increases the risk of type 2 diabetes, metabolic dysfunction–associated steatotic liver disease (MASLD), stroke, chronic kidney disease, and certain cancers, all of which have major health and economic implications (17, 18). Of the component conditions, diabetes is of note, as it alone affects ~9% of the world's population (>422 million adults), and in the United States 30 million adults (9.4% of the population) are estimated to be diabetic, with a further 84 million (34% of population) being prediabetic (16).

These dramatic changes in metabolic health are occurring alongside a parallel and alarming decline in human fertility/fecundity and increase in reproductive health problems (19–21). The human fertility rate declined from 5 to 2.5 births per woman between 1960 and 2015 (22), and worldwide an estimated 60–80 million couples seek medical help with reproduction (23). In the United States, the percentage of women of reproductive age that have difficulty becoming pregnant has risen from 8% to 12% since the early 1980s (24). The problems do not lie predominantly within one sex, as both male (25) and female (24) fertility are in decline. In women, the incidence of ovarian dysgenesis syndrome (26), which encompasses premature ovarian failure, ovarian cancer, polycystic ovarian syndrome (PCOS), and reduced fertility, has risen dramatically (27). In men, sperm counts declined globally by up to 50% between 1938 and 2011 (28, 29). A cross-sectional population-based study also reported that semen quality in 25% of Finnish and 35% of Danish men (30) has fallen below the World Health Organization reference for normal fertility ($30–55 \times 10^6$ sperm/mL) (31). These changes in sperm quality/output are occurring alongside increased congenital male reproductive malformations (e.g., cryptorchidism and hypospadias) and testicular cancer, which exist under the umbrella of testicular dysgenesis syndrome (TDS), the reported incidence of which has increased over the last 50 years, particularly among men of European descent (31).

The etiology of impaired metabolic and reproductive health is undoubtedly multifactorial. Socioeconomic factors, diet, and a sedentary lifestyle impact metabolic health (32), and societal changes, such as increased maternal age at first birth (33), are likely to contribute to the observed decline in fertility. The interrelationship between adverse metabolic and reproductive outcomes [for example, the association between MetS and both decreased male (34) and female (35) reproductive health and fertility] and the link between MetS and the most common form of female reproductive endocrine infertility, PCOS (36, 37), beg the question of whether common physiological mechanisms underlie or link changes in reproductive and metabolic health.

3.1. EC Exposure and Declining Human Metabolic Health

Baillie-Hamilton (38) reported associations between exposure to ECs found in pesticides, solvents, plastics, and flame retardants and increased weight gain. Additional ECs have been recognized subsequently to alter hormonal pathways that regulate lipid metabolism, stimulate adipocyte differentiation, and predispose individuals to obesity and/or related metabolic disorders. Such ECs can be classified as obesogens and/or metabolism-disrupting chemicals (39–41). In 2015, a review published by the Endocrine Society found strong evidence for ECs' role in the etiology of metabolic diseases (42), and several subsequent epidemiological studies have provided intriguing links between ECs and metabolic disease. For example, diabetes has been linked to DDT and its metabolite DDE, dioxins, PCBs, and BPA exposure (43–45). The latter chemical has also been associated with obesity and insulin resistance (46) and MASLD (47). Phthalates (e.g., DEHP) have also been linked to MetS (48–50). Intriguingly, exposure to some ECs, including phthalates (51), BPA (52), multiple PCB congeners (53), PFAS chemicals (54), and organochlorine pesticides (55), may also contribute toward increased CVD risk. In terms of everyday exposure to ECs, recent evidence has highlighted air pollution as a possible contributory factor to metabolic diseases. Air pollutants include mixtures of ECs such as benzo(a)pyrene (the main marker of PAH presence), PCBs, sulfur dioxide, nitrogen dioxide, carbon monoxide, organic compounds (organic solvents and dioxins), and heavy metals, often produced by transport and industrial processes. Air pollution is associated with diabetes, CVD, and MASLD (56, 57). Together, these studies suggest that a diverse array of ECs may individually, or collectively, play a significant role in the programming and pathophysiology of various metabolic diseases.

3.2. EC Exposure and Declining Human Reproductive Health

Several specific ECs have been linked with female reproductive health problems (58), TDS, and a decline in male reproductive function (42, 59). Estrogenic ECs (60) such as bisphenols (e.g., BPA) (61) and per- and polyfluorinated alkyl substances (PFAS) (62) are directly associated with breast cancer; however, causality has not been established (63). EC exposure has also been indirectly linked with breast cancer risk via effects on other aspects of female reproductive health, including precocious onset of puberty (60). Although female infertility is a major and growing concern, due to factors such as advanced reproductive age, 15–30% of cases remain unexplained. However, recognized contributory factors such as premature ovarian insufficiency, endometriosis, and PCOS have all been associated with EC exposure (64–67). Exposure to phthalate esters variably affects puberty onset; some studies have shown premature thelarche (68) and early/precocious puberty in girls (69–71), whereas others report delayed pubarche without thelarche (72) or no effect (73).

As with the effects in females, the relationship between adult EC exposure and male reproductive health and function is similarly complex. For example, BPA exposure has been negatively associated with sperm concentration, sperm motility, and total sperm count (74–76). Phthalate [DEHP, di(*n*-butyl) phthalate (DBP)] exposure has an inverse relationship with anogenital distance (AGD) (77); a negative association with sperm concentration and sperm motility (78); a positive association with semen volume, progressive motility (78), and anopenile distance (79); and no association with semen quality (80). Serum concentrations of PCB congeners PCB-118 and PCB-77 have both negative and positive associations with semen volume and progressive motility (81). DEHP and PCB-153 reduce human sperm motility and increase DNA fragmentation (82), and PBDEs are both without effect on semen parameters (80) and negatively associated with sperm concentration, total sperm count, and progressive motility and viability (83).

Fertility in both sexes has been reported to be negatively impacted by air pollution (84), potentially due to ECs such as benzo(a)pyrene (the main marker of PAH presence), PCBs, sulfur dioxide, nitrogen dioxide, carbon monoxide, organic compounds (organic solvents and dioxins), and heavy metals, which are produced by transport and industrial processes. In males, industrial air pollution is associated with decreased fertility (85, 86) and reduced sperm quality (number and motility) (84, 87–89). In females, air pollution can lead to reduced odds of positive in vitro fertilization pregnancy outcomes (90) and is associated with increased risk of stillbirth (91). Additionally, exposure to particulate matter, also found in air pollution, is associated with reduced fecundability (92), decreased antral follicle count, and anti-Müllerian hormone levels, suggesting a negative impact on ovarian reserve (93). However, as with many of the studies of the effects of individual ECs, the relevance of these studies relative to real-life EC exposure is limited by heterogeneity among studies, low numbers of participants, the small numbers of pollutants analyzed, and analytical methods.

3.3. Programming of Metabolic and Reproductive Health Outcomes by Developmental EC Exposure

The concept that the early developmental environment can program adult disease is well established (94) and supported by both human epidemiological and animal studies (95–98). Research into the developmental origins of health and disease (DOHaD) has focused largely on maternal nutrition and/or stress experienced during pregnancy and consequences for offspring cardiovascular and metabolic health (99–103). Studies also indicate that reproductive development and function can be affected (104). DOHaD-related health effects may be detected in the fetus and persist until adulthood. Other effects may not become apparent until adulthood. Irrespective of timing, these effects usually occur due to epigenetic programming (103, 105) (discussed below). Emerging evidence also suggests that poor metabolic and reproductive health arise as a consequence of events experienced by our parents, grandparents, or even earlier generations (106, 107).

A well-publicized example of EC-induced DOHaD relates to the gestational exposure of women to the synthetic estrogen diethylstilbesterol (DES), as their offspring have a high lifetime risk of a range of adverse health outcomes including breast cancer (108). Basic clinical and epidemiological evidence also suggests that TDS may often originate during fetal life (109) as a consequence of in utero exposure to ECs (110). Although establishing the causation of EC exposure effects on health can be challenging in human studies, some of the most compelling evidence comes from cases where developmental EC exposure has occurred following an occupational or environmental accident. For example, men who were perinatally exposed to dioxin in Seveso, Italy (111), or prenatally exposed to PCBs and polychlorinated dibenzofurans in Taiwan (YuCheng accident) (112) exhibited reduced semen quality as adults. Other studies have reported increased risks of genital malformations in children of workers occupationally exposed to pesticides (113), and high incidences of cryptorchidism are observed in geographical regions with intensive agriculture (114) or high levels of industrialization (115). Furthermore, maternal phthalate exposure is associated with impaired Leydig cell function (116). Developmental exposure to obesogenic ECs has also been linked to latent effects on metabolic health (117, 118). For example, maternal exposure to BPA and phthalates resulted in altered offspring growth and body mass index in childhood (119), and developmental perfluorooctanoic acid exposure may reduce fetal growth (120).

Finally, women who smoke during pregnancy provide a natural human paradigm to determine the latent effects of exposure to EC mixtures. Cigarette smoking increases the risk of various adverse pregnancy outcomes such as miscarriage, placenta previa, preeclampsia, and premature delivery (121). Importantly, chemicals contained in cigarettes can cross the placenta and have a negative impact on the fetus at the genetic and cellular level. Consequently, maternal smoking during pregnancy has increased our understanding of the long-term effects of early-life exposure to EC mixtures on offspring health. Longitudinal studies that have followed the offspring of mothers exposed to cigarette smoke have demonstrated that it affects reproductive and metabolic health of offspring both as children and as adults. For example, males whose mothers smoked during pregnancy have reduced semen quality and testis size in adulthood (122, 123), and female offspring exhibit altered ovarian development (124) and reduced fecundability (125). In parallel, maternal smoking is linked to intrauterine growth restriction and low birth weight for gestation age (126), as well as childhood and adult obesity (127, 128) and CVD risk in later life (129, 130). Given that exposure to individual chemicals (e.g., perfluorooctanoic acid) (131) and chemical mixtures (cigarette smoke) alters fetal growth, and that intrauterine growth restriction is a known risk factor for CVD in later life according to DoHaD, it is possible that the mechanisms that underlie fetal programming of health are sensitive to EC exposure as well as other maternal factors and that these share common features.

In summary, there is some compelling evidence linking human exposure to ECs, either as adults or during development, with alterations in metabolic and/or reproductive health. Many studies have focused on either accidental or occupational exposure to high levels of ECs or have reported associations between individual or limited mixtures of chemicals and metabolic and reproductive outcomes. However, by their very nature, such studies are associative and do not establish causation. Outcomes have also been variable and sometimes confounded by small study populations and other lifestyle variables. To complement human studies, therefore, use of prospective, controlled animal models of individual and mixed EC exposure has been necessary.

4. MAMMALIAN MODELS FOR THE STUDY OF ECs

Animal models provide opportunities to assess the effects of dose and timing of EC exposure and to interrogate mechanisms of action of individual and combined ECs across the life course. Even with such paradigms, however, modeling real-life mixed EC exposure remains challenging. Importantly, EC exposure and effects of EC exposure may be influenced by species-specific differences in absorption, metabolism, and elimination of ECs, and in the duration of developmental stages (e.g., differences in gestation length), which affects the relative duration of periods of EC exposure and could affect the nature of their effects. The use of multiple models, and comparison across animal models, allows the individual strengths of each model to be utilized and accounted for and significantly advances our understanding of the possible health effects of EC exposure.

4.1. Companion Animals as Sentinel Species

Because companion animals share our home environment, cats and dogs could be used as sentinel species for the effects of human EC exposure (132, 133). As with humans, companion animal EC exposure occurs through food, air, dust, and water and has been linked to both adverse reproductive and metabolic outcomes (133). In cats, high levels of ECs such as PBDEs (flame retardants) are thought to result from contact and grooming and have been linked with thyroid dysfunction (134), metabolic disorders, and cancer (135), whereas other ECs, such as PFAS, have been linked to obesity (136). In dogs, a 30% decline in progressive sperm motility has been reported over a 26-year period alongside an increased incidence of cryptorchidism (137, 138). These canine data are of note because they were obtained from a single laboratory and thus lack some of the inherent confounders in human meta-analyses of the effects of ECs on TDS. In addition, ECs have been detected in dog testes at concentrations able to inhibit sperm motility (dog and human), and as with humans, testicular EC profiles vary geographically (82, 139). Some comparative studies done with companion animal species have reported that metabolic disease can be linked with high PFAS concentrations in both cats and dogs (133).

4.2. The Sheep Biosolids Sentinel and Animal Model

Another animal model that reflects human EC exposure is the biosolids-exposed sheep model. Biosolids is the solid waste generated in wastewater treatment plants. It is a nutrient-rich organic matter and is used globally as an agricultural fertilizer. Indeed, biosolids use is recommended by the US Environmental Protection Agency and through the European Sewage Sludge (biosolids) Directive. Approximately 55% of the 18 million dry metric tonnes of treated sewage sludge produced annually in the United States is applied to agricultural land (140). Because biosolids originates from human activity, it contains a broad mixture of ECs, including those derived from personal-care products, pharmaceuticals, pesticides, flame retardants, detergents, BPA, PCBs, PBDEs, PAHs, organochloride pesticides, and pharmaceuticals and ECs in waste from industrialized manufacturing processes (141–145). Application of biosolids to land results in increased EC concentrations in soil (143–145) and in tissues and blood samples collected from sheep grazed on biosolids-treated pastures (146–149). It therefore constitutes an exposure paradigm that reflects the human exposome and has inadvertently provided an excellent model of real-life human EC exposure. Over 25 years, this model has been used in a series of studies investigating the impact of gestational exposure to EC mixtures across different life stages (fetal, neonatal, prepubertal, adult) of the offspring. Initial investigations focused on the reproductive axis (150–158), but studies have also documented effects on behavior (159), bone density (160), thyroid structure (160), liver function (161), and more recently metabolic parameters in both exposed mothers and their offspring (162–164).

Relative to reproductive health, exposure of male fetuses to ECs by maternal grazing on biosolids-treated pasture either throughout or for an 80-day period during early, mid-, or late gestation results in an abnormal testicular phenotype, a characteristic of which is reduced numbers of Sertoli cells (158). The developmental and transcriptomic changes observed in gestational day (GD) 140 male fetuses indicate an overall antiandrogenic effect (165, 166). In female fetuses, GD80 and GD110, there is a reduction in transitional ovarian follicles (158, 166), and EC exposure for 80 days during mid- and late gestation appears to result in greater effects, with animals exhibiting a reduced AGD indicative of an overall androgenization effect (167–169). Male and female fetal lambs exposed to ECs via biosolids, either throughout or during the final third of gestation, have altered expression of drug-metabolizing genes. This could account for greater effects of transient versus continuous exposure across gestation observed in various outcomes in this model (166, 170). EC exposure via biosolids in both male and female fetuses (GD140) also alters the gonadotropin-releasing hormone neurosecretory system along with other key neuroendocrine regulators (e.g., kisspeptin, galanin receptors) in the hypothalamus and pituitary, thus illustrating hypothalamic–pituitary–gonadal axis sensitivity at all three levels (152, 153, 168). When considering fetal effects of ECs, these could be mediated indirectly through the mother, as EC exposure induces changes in the maternal metabolome, in her steroid profile, and in the expression of mediators of oxidative stress (155, 171).

In later life, pubertal timing is affected in lambs born to mothers exposed to biosolids immediately prior to and through gestation; it is advanced in male but delayed in female offspring (164). Furthermore, when the EC exposure period was extended by the grazing of lambs exposed during gestation through to puberty, a subset of the adult males exhibited testicular abnormalities, including Sertoli cell–only tubules and reduced numbers of germ cells (162, 163). Beyond puberty, 11-month-old (adult) male offspring exposed in utero exhibited two metabolic profiles indicative of different degrees of susceptibility to EC exposure (150, 157). In summary, these extensive studies illustrate that exposure to biosolids during gestation adversely affects fetal, neonatal, pubertal, and adult reproductive and/or metabolic function (**Figure 1**).

4.3. Rodents

Historically, most EC exposure studies have used rodent models (172). This reflects their ease of management, short gestation length, controlled genetics, the capacity to explore chemical effects at the population level, transgenerational studies, and the use of transgenic and knockout mice

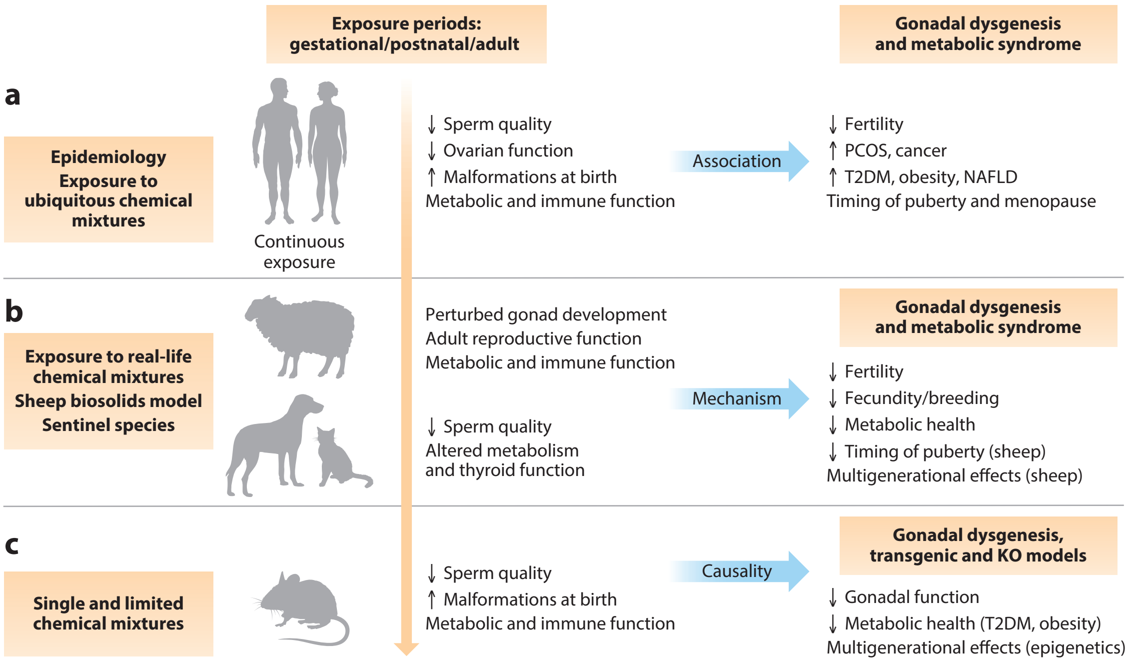

Figure 1

Mammalian models for the assessment of EC effects on reproductive and metabolic function. (*a*) Human studies are largely epidemiology based; temporal trends in gonadal dysgenesis and metabolic syndromes have been reported. (*b*) Real-life chemical effects can be explored in a highly characterized sheep model of exposure to EC mixtures present in biosolids derived from human sewage/effluent. Companion animal sentinels (i.e., dog, cat) reflect reproductive and metabolic changes in the human. Both models have shown a decline in fertility, fecundity, and puberty timing (sheep), and multigenerational studies in sheep are ongoing. (*c*) Rodent models of gonadal dysgenesis, transgenics, and KO strains are excellent tools to investigate causality, including multigenerational effects. Abbreviations: EC, environmental chemical; KO, knockout; NAFLD, nonalcoholic fatty liver disease; PCOS, polycystic ovarian syndrome; T2DM, type 2 diabetes mellitus.

to explore the role of EC-sensitive genes in biological pathways. Despite some obvious caveats around the use of polytocous rodents when extrapolating EC impacts to humans, the contribution of rodent studies to the understanding of EC action is substantial.

Given that EC exposure is associated with TDS in humans (see Section 3), some earlier studies focused on mechanisms underlying a comparable TDS phenotype in a murine model. Landmark studies identified an early programming window (gestational days 15.5–19.5) in male rats, which corresponds to the period between 8 and 14 weeks in humans, as during this period exposure of rats to the antiandrogen flutamide induces both cryptorchidism and hypospadias (173), characteristics of the TDS phenotype widely reported in the human. Intriguingly, exposure of the female rat fetus to androgens during this window increased AGD length comparable to that in males, illustrating a similar masculinization programming window to androgens in utero. In humans, TDS is associated with reduced AGD. Notably, in the rat, exposure of the male fetus to the plasticizer DBP is associated with reduced testosterone and AGD only when exposure occurs during the equivalent androgenization window outlined above (165, 174, 175). Rodent models have, therefore, allowed us to interrogate the effects of ECs and EC mixtures during periods of known developmental sensitivity (165, 176).

Rodent models have also been used to identify developmental periods of sensitivity and potential underlying mechanisms involved in premature ovarian insufficiency, reduced fertility, PCOS, and endometriosis. Because the timing of fetal ovarian development in the rodent differs to that in the human, with early follicle development occurring postpartum, this presents some experimental opportunities, but with caveats. Such models provide access to follicle development stages that occur prenatally in the human. However, the route of EC exposure is via placental transfer in humans but is predominantly oral in postnatal rodents. Complementary in vitro approaches using excised and cultured ovaries to study the effects of mixed EC exposure on follicle development have been underused.

Rodents have also been instrumental in determining EC effects linked to metabolic dysfunction (177), obesity and type 2 diabetes mellitus, and MetS (178, 179). One specific example is that of perinatal BPA exposure, which has been shown to induce changes in DNA methylation (candidate gene and global). Specifically, pathway analyses indicate changes in DNA methylation of metabolic and neural signaling genes (179). Knockout mice provide ideal tools for elucidating reproductive or metabolic mechanisms. For example, comparisons of $Ahr^{+/+}$ to $Ahr^{-/-}$ mice clearly demonstrate the adverse consequences of dioxin exposure on liver metabolism (177). That is, hepatotoxicity was dependent on the presence of the Ahr receptor. Endocrine-/metabolism-disrupting chemicals can interfere with several additional signaling pathways for which knockout mice are available. These include the estrogen, retinoid X, androgen, and several other receptor-mediated pathways (180). Thus, rodents are ideal experimental tools to delineate molecular mechanisms associated with effects of ECs on reproduction and metabolism (**Figure 1**).

5. MECHANISMS OF ACTION

The deleterious effects of ECs on both metabolic and reproductive function can be mediated centrally (i.e., within the hypothalamic–pituitary axis) and/or locally (i.e., within specific tissues and organs). For example, the homeorhetic regulation of basal metabolic rate, appetite, and energy metabolism by thyroid hormones is mediated both centrally and peripherally (including the gravid uterus) (181) (**Figure 2**). The consequences of in utero exposure and their contributions to the DOHaD effects of ECs are discussed earlier in this article. EC-disrupted thyroid hormone–directed signaling in the liver largely affects lipid and glucose metabolism (182), whereas perturbed thyroid hormone–directed signaling in the pancreas mostly influences

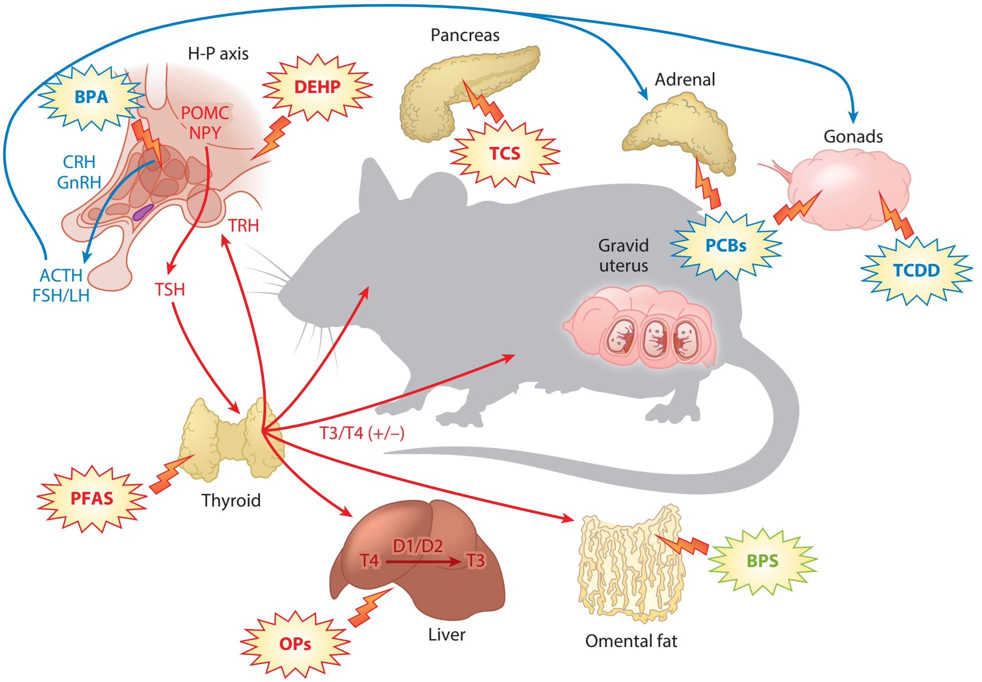

Figure 2

Targeted effects of ECs impact multiple organ and tissue systems, including the gravid uterus in pregnant females. Depending on exposure timing and duration, these effects can be limited to founder (F0) individuals or extend to F1 and subsequent generations following in utero exposure. Illustrative examples are provided firstly for effects of specific ECs on the regulation of pleiotropic thyroid hormones (181) involved in the homeorhetic and homeostatic regulation of cellular metabolism and appetite. These effects can occur centrally (e.g., DEHP) (264, 265), acting on neural networks (e.g., POMC, NPY) controlling food intake or on TRH, which regulates the pituitary release of TSH. ECs can also act directly on the thyroid [e.g., PFAS (266)] to affect T4 and T3 production or locally at target organs and tissues [e.g., OPs in the liver (182) and TCS in the pancreas (183)]. Importantly, many of these local effects include the dysregulation of iodothyronine deiodinases (e.g., D1 and D2) that coordinate T4-to-T3 conversions in a tissue-specific manner. Also depicted are EC effects (e.g., BPA) on the hypothalamic–pituitary production and release of gonadotrophins (FSH/LH) (267) and ACTH (268), which act on gonads and adrenal glands, respectively, to disrupt function and dysregulate steroid production. ECs can also have direct effects on tissues, which, although apparent from in vivo studies [e.g., effects of dioxins (TCDD) on ovarian function (185)], are best demonstrated by either cell or organ culture experiments [e.g., PCBs on gonadal and adrenal cells (269) and BPS on adipocytes (270)]. Blue arrows: H-P hormone actions on reproductive tissues. Red arrows: target organs/tissues affected by pleiotropic thyroid hormones. Bursts depict action of environmental chemicals, which can be either direct or indirect (acting via the H-P axis or the thyroid gland). Abbreviations: ACTH, adrenocorticotrophic hormone; BPA, bisphenol A; BPS, bisphenol S; CRH, corticotropin-releasing hormone; DEHP, diethylhexyl phthalate; EC, environmental chemical; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; H-P, hypothalamic-pituitary; LH, luteinizing hormone; NPY, neuropeptide Y; OPs, organophosphates; PCBs, polychlorinated biphenyls; PFAS, polyfluoroalkyl substances; POMC, pro-opiomelanocortin; T3, triiodothyronine; T4, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCS, triclosan; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

glucose-stimulated insulin secretion (183). Likewise, ECs can affect both positive and negative feedback mechanisms operating within the hypothalamic–pituitary–gonadal axis to affect reproductive development/function (152, 153) as well as autocrine and paracrine mechanisms within gonads that regulate steroidogenesis and germ-cell maturation (184, 185) (**Figure 2**).

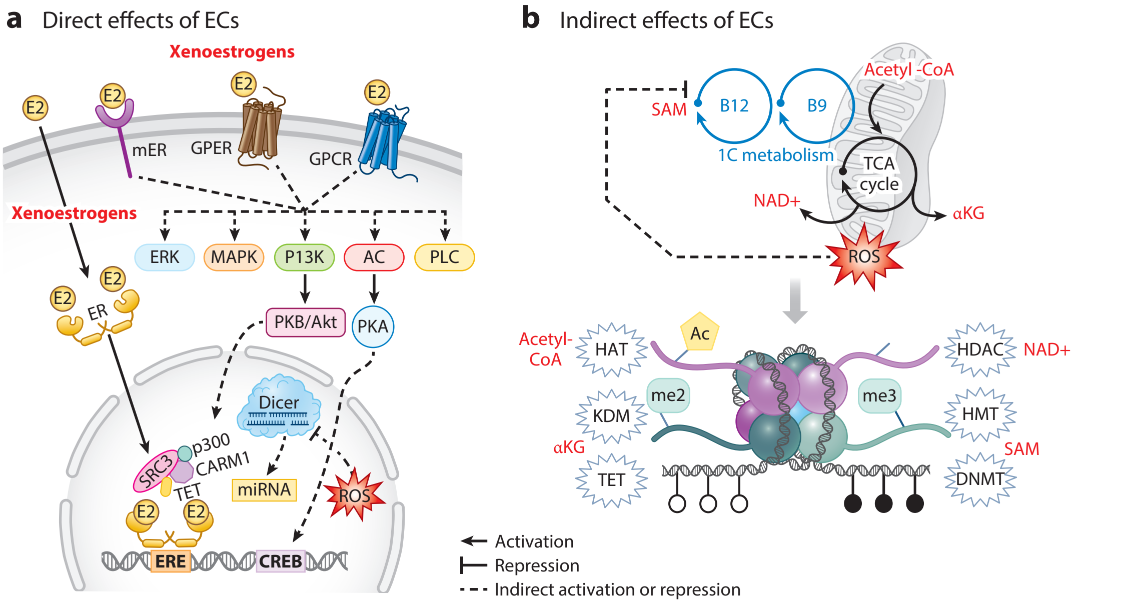

Figure 3

Examples of (*a*) direct and (*b*) indirect modes of action for ECs in the epigenetic regulation of gene expression. (*a*) Direct actions of ECs are best characterized for nuclear receptor superfamily members; the example presented is for the ER depicting genomic and nongenomic signaling related to transcriptional activation. Xenoestrogens (e.g., BPA, DES) can substitute for estrogen binding to cytoplasmic/nuclear receptors at gene-promotor ERE and could thus epigenetically alter the coordinated recruitment of chromatin modifiers and coactivators (e.g., SRC3, p300, CARM1, and TET) required for transcriptional activation, further modified by the actions of miRNAs (e.g., miR-494). Nongenomic epigenetic actions of xenoestrogens can be mediated by any combination of three membrane-bound receptors (i.e., mER, GPER, or GPCR) to permanently alter intracellular protein kinase cascades involving ERK, MAPK, P13K, AC, and/or PLC signaling. The relative contribution of these pathways and details regarding EC-induced heritable changes in chromatin configuration related to estrogen signaling are the subject of ongoing investigations by several groups. (*b*) Indirect actions of ECs can arise due to modifications in intermediary metabolism that alter the availability of metabolites that serve as substrates or cofactors for enzymes involved in chromatin modification. These include acetyl-CoA-dependent HATs, NAD^+-dependent HDAC (e.g., Sirtuins), SAM-dependent HMT and DNMT; and αKG-dependent histone (KDM) and TET demethylases (271, 272). Many genes encoding enzymes involved in 1C, as well as associated epigenetic regulators, are modulated either directly or indirectly by androgen or estrogen receptors (as depicted in panel *a*) (202). Exposure to ECs such as heavy metals and EDCs can also lead to the excessive generation of ROS, which, in turn, can (*i*) induce DNA breaks and dysregulate associated base-repair mechanisms; (*ii*) directly impede/alter chromatin modification (e.g., carbonylation and glutathionylation) as well as perturbing 1C metabolism; and (*iii*) inhibit Dicer activity, (*a*) thus impairing miRNA maturation (217). Open and solid lollipops represent unmethylated and methylated CpGs, respectively, on DNA. Abbreviations: αKG, α-ketoglutarate; 1C, one carbon; B9, folate; B12, cobalamin; BPA, bisphenol A; CARM1, coactivator-associated arginine methyltransferase 1; DES, diethylstilbesterol; DNMT, DNA methyltransferase; EC, environmental chemical; EDC, endocrine-disrupting compound; ER, estrogen receptor; ERE, estrogen-response element; ERK, extracellular signal regulated kinase; GPCR, G protein–coupled receptor; GPER, G protein–coupled estrogen receptor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; KDM, lysine demethylase; MAPK, mitogen-activated protein kinase; miRNA, microRNA; NAD, nicotinamide adenine dinucleotide; P13K, phosphoinositide 3-kinase; PKA, protein kinase A; PKB, protein kinase B; PLC, phospholipase C; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; SRC3, steroid receptor coactivator-3; TCA, tricarboxylic acid; TET, ten-eleven translocation.

One mode of action of ECs is to mimic the behavior of natural hormones. Xenoestrogens such as BPA, for example, mimic the effects of estradiol by binding to both nuclear (i.e., estrogen, androgen, and glucocorticoid) and membrane (i.e., GPER) receptors (**Figure 3**). In so doing, they interact with several transcription factors (e.g., PPARγ, C/EBP) to alter adipose and liver homeostasis (186). The antiandrogen effects of BPA also operate in this way, leading to impaired testicular

EPIGENETIC PROGRAMMING

The term epigenetics refers to heritable, yet reversible, changes in gene function that arise in the absence of an alteration to DNA sequence. These changes relate principally to covalent modifications in DNA and associated proteins that are directed to an extent by noncoding RNAs (197, 198). Heritable in this context relates to the mitotic transmission of such modifications from parent to daughter cells during cell division. It follows that epigenetic processes are central to directing cell fate and lineage determination during postfertilization development. However, as discussed later, there is some debate concerning the extent to which modifications can be propagated through meiosis (i.e., intergenerationally) in mammals due to the double wave of epigenetic reprogramming that occurs during early embryogenesis and later in primordial germ cells (199). Notwithstanding, sweeping epigenetic modifications occur around the time of conception when, during syngamy, two terminally differentiated cells (i.e., the sperm and egg) give rise to a totipotent zygote. Epigenetic memory is important for guiding cell fate during later developmental stages, and this is achieved partially through preference for concordant DNA methylation (200). However, early embryonic cells operate with reduced fidelity of methyl-group transfer, rendering them more susceptible to environmentally induced perturbations in DNA methylation. Consequently, it has been argued that the periconceptional period is the development stage most sensitive to potential modifications due to extraneous factors such as environmental chemicals (201, 202), although recognized later stages of development [e.g., the masculinization programming window (165)] may also be epigenetically sensitive to specific agents.

physiology and reduced spermatogenesis. ECs also act indirectly by increasing oxidative stress and DNA damage, leading to apoptosis. Many serve as oxidants and impact on mitochondrial function, generating reactive oxygen species and other reactive compounds (187). Oxidative stress during pregnancy can lead to birth defects (188), impairing reproductive function, cardiometabolic health, and vascular dysfunction in CVD (189–191). Apoptosis and inflammation are hypothesized to play a role in these processes. For example, the apoptotic/inflammation regulatory molecule NFκB has been implicated in both testicular germ cell apoptosis (192, 193) and inflammation involved in endothelial damage and atherosclerosis (194) in response to chemical exposure. However, more fundamental mechanisms of action underpinning the harmful effects of ECs pertain to the epigenetic regulation of gene expression, in particular, long-term (including transgenerational) adverse developmental effects observed following EC exposure during pregnancy and/or infancy.

5.1. EC-Induced Epigenetic Effects

A series of systematic reviews published in the last decade have summarized comprehensively the epigenetic consequences of EC exposure at different stages of development (i.e., in utero, during infancy, and in adulthood) (172, 173, 175, 195, 196; see the sidebar titled Epigenetic Programming). These reviews indicated that most studies related to humans and rodents and the reported effects were associative in nature (particularly in humans). Changes in DNA methylation were the most frequently investigated epigenetic modifications, although recent studies have begun to explore associations with noncoding (nc)RNAs (particularly small ncRNAs) and post-translational modifications to histones. Despite heterogeneity between cited studies, EC exposure, including air pollution and cigarette smoking during pregnancy, along with an extensive list of recognized ECs including heavy metals, POPs (e.g., dioxins, PCBs), and known carcinogens, consistently induced epigenetic modifications to chromatin in a range of cell and tissue types.

More recently, there has been an attempt to identify homologous DNA methylation reprogramming following lead and phthalate (DEHP) exposure in mice and humans, with the aim of enhancing the translational value of mice in toxicology studies (203). Mouse databases originating from the National Institute of Environmental Health Sciences–sponsored TaRGET II program (204) were compared to four human cohorts with previously published DNA methylation data. In mice, lead and DEHP exposure during pregnancy and up to weaning led to a significant number of differentially methylated genes (∼750) in both male and female offspring, which corresponded to ∼35,000 differentially methylated cytosines on the human EPIC array. Although there was little overlap with reported differentially methylated regions in mice, the regions that did overlap included three imprinted loci (i.e., *KCNQ1*, *CDKNIC*, and *CMTM1*) (discussed later).The main conclusion to be drawn from this study is that, although animals serve as great models to establish and understand the epigenetic basis of adverse phenotypic effects following exposure to ECs, they do not represent direct comparators for humans. Instead, the power of animal studies (rodents in particular) lies in their ability to go beyond association analyses to undertake prospective interventional studies (e.g., gene knockout/in), to establish causality and mode of action, and to assess long-term (including transgenerational) epigenetic and phenotypic effects of EC exposure. To this end, transgenic mice have been used to explore aspects of germline epigenetic inheritance in a paternal obesity paradigm (205–207), and the adverse metabolic effects to offspring of feeding high-fat diets to sires were recapitulated when purified small ncRNAs (identified following sperm analyses) were injected into zygotes prior to embryo transfer (208). However, these approaches have yet to be exploited in the context of EC exposure.

5.2. Mechanisms Underlying EC-Induced Epigenetic Modifications

ECs could induce epigenetic modifications to chromatin via several means, although they have yet to be investigated fully. From what is known, mechanisms can be categorized as either direct or indirect and, related to this, global or gene specific in nature (209) (**Figure 3**). However, it is important to distinguish chromatin-based mechanisms that transiently facilitate transcription from those that are genuinely epigenetic; the latter term infers that any modifications to chromatin are heritable (210; see the sidebar titled Epigenetic Programming). Steroid receptors, for example, can transiently activate transcription through recruitment of several coregulators to facilitate histone modification, chromatin remodeling, and general transcription machinery stabilization (211). ECs can induce heritable (epigenetic) modifications to these processes, but the precise mechanisms involved have not yet been established.

A case in point is the estrogen receptor (ER), a subclass of the nuclear receptor superfamily of proteins (212), which in most cases binds directly (or indirectly) via AP-1 or SP-1 transcription factors to estrogen-responsive elements in DNA of target genes (213) (**Figure 3*a***). Upon ER binding, the process of rendering chromatin more accessible to transcription factors generally involves the sequential recruitment of a cohort of chromatin modifiers and coactivators commencing with SRC-3, which recruits p300 (a histone acetyltransferase) and CARM1 (coactivator-associated arginine methyltransferase 1). This is followed by either passive or active demethylation of DNA, the latter step involving TET (ten-eleven translocation) enzymes. A further layer of complexity involves actions of small ncRNAs (e.g., miR-494) that can modulate these processes. However, endogenous estrogens and xenoestrogens (e.g., BPA, DES, PCBs) can also bind to membrane-bound ERs, and so epigenetic modifications to chromatin can be induced via both genomic and nongenomic pathways, although the relative importance of each is uncertain (214). Although the recent development of transgenic mice lacking either membrane or nuclear ERs will provide valuable insights in this regard (215), our understanding of how these events lead to heritable changes to chromatin remains to be determined.

Indirect effects of ECs can be mediated in part through actions on intermediary metabolism operating, for example, in and around the mitochondrion (**Figure 3*b***). This can arise due to

EC-induced oxidative stress and/or modifications in levels of metabolites that serve as substrates or cofactors for enzymes involved in the epigenetic modification of chromatin and/or coding and ncRNAs (216). For example, the excessive generation of reactive oxygen and nitrogen species following EC exposure can interfere with the activity of enzymes involved in chromatin methylation and acetylation, thus inducing DNA damage and activating base-repair mechanisms (217). It can also lead to the oxidation of enzymes involved in one carbon (1C) metabolism or induce excessive glutathione production, thus depleting *S*-adenosyl methionine and chromatin methylation. Recent examples of indirect effects include exposure to the carcinogenic and neurotoxic element cobalt, which was found to downregulate the expression of the m^6A demethylase ALKBH5, leading to an enrichment of both hypermethylated and hypomethylated transcripts linked to neurodegenerative diseases (218). Confirming a nonspecific role for 1C metabolism, folic acid supplementation of pregnant rats exposed to a mixture of POPs mitigated observed adverse effects on sperm microRNA profiles in offspring over four successive generations (219).

5.3. Genomic Imprinting

Although most mammalian genes are expressed by both parental alleles, a small number of genes [recent estimates for the mouse list $\geq$388 (220)] are expressed by only one allele in a parent-of-origin-specific manner (see the sidebar titled Mechanisms Directing Mono-Allelic Gene Expression). These are referred to as imprinted genes. The importance of genomic imprinting in the context of mammalian development resides in the fact that uniparental embryos generally fail to develop much beyond implantation. Errors in genomic imprinting result in aberrant placental and fetal development in mammals (221, 222) but can also lead to lifelong disorders, including Angelman, Prader–Willi, and Beckwith–Wiedemann syndromes in humans (222) and related large offspring syndrome in cattle and sheep (223–225).

MECHANISMS DIRECTING MONO-ALLELIC GENE EXPRESSION

Parent-of-origin-specific gene expression is regulated by the inheritance of differentially methylated imprinting control regions (ICRs), together with other germline differentially methylated regions (DMRs). The establishment of these ICRs/DMRs during gametogenesis is carefully choreographed, with the transient presence of histone variants [e.g., histone 3 lysine 4 di/tri-methylation (H3K4me2/3), H3K27me3, and H3K36me3 (220, 226–228)] operating in cohort with local transcriptional events specific to male and female germlines. These serve to direct allele-specific methylation actioned by the methyltransferase complex DNMT3A-DNMT3L (229). For specific imprinted genes, PIWI-interacting RNAs, along with other short- and long-noncoding RNAs, serve as part of the transcriptional machinery that regulates de novo methylation at DMRs (230). Normally, ICRs/DMRs escape the wave of genome-wide demethylation that occurs during preimplantation stages of embryo development (231). This arises through the combined actions of the maintenance DNA methyltransferase and its partner, ubiquitin-like with PHD and ring finger domains 1, together with two Krüppel-associated box domain zinc-finger proteins. These factors bind specifically to the methylated allele, thus protecting it from demethylation while directing methylation to the nascent allele arising following DNA replication during cell division (232–234). In addition, evidence is emerging for a key role of long-terminal-repeat retrotransposons (LTR) in both canonical and noncanonical (i.e., DNA methylation–independent) imprint establishment during gametogenesis and maintenance in embryonic- and somatic-cell lineages, although noncanonical imprinting appears to be transient in nature (228). Nevertheless, LTR-guided imprinting imposes an additional level of complexity, particularly in outbred species such as humans, as these sequences are highly polymorphic and therefore likely to contribute to molecular and phenotypic differences observed between individuals.

Several putative molecular targets for ECs could lead to the erroneous expression of this subset of developmentally important genes. Indeed, rodent studies have identified effects of several ECs, including BPA; phthalates (e.g., DEHP); 2,3,7,8-tetrachlorodibenzo-p-dioxin; and the pesticide vinclozolin on DNA methylation and expression of imprinted genes. However, reported outcomes (including phenotypic) are variable and mechanistic details sparse, focusing mostly on canonical modes of action (reviewed in 235). In these studies, the imprinted genes concerned were preselected and include those for which previous evidence of more general environmentally induced dysregulation in methylation and expression exists (e.g., *Igfr2*, *Peg3*, *Snrpn*, *Igf2/H19*) (232, 233). Several factors likely contributed to the variability in effects observed, including rodent strain (236), specific ECs concerned, exposure timing (i.e., gametogenesis versus early embryogenesis versus later stages of gestation and/or infancy), dose (pharmacological versus physiological), and route of administration (e.g., oral versus hypodermal). Importantly, to date, most rodent studies investigating EC effects on genomic imprinting have focused on single chemicals or small mixtures of chemicals. Also, it will be necessary to establish the full extent of sex specificity in responses (at both a molecular and physiological level) for these genes and to determine both canonical and noncanonical mechanisms of dysregulated genomic imprinting.

Investigations into the effects of EC-induced perturbations in imprinted genes in large, outbred mammalian species are limited. However, several studies in humans, spanning a wide range of geographical locations (and ethnicities), have reported errors in genomic imprinting following EC exposure during pregnancy (235). Although observational and associative in nature [being mostly linked to cord blood or urinary levels of phthalates or BPA (237–240)], these studies demonstrate that real-life EC-induced errors in genomic imprinting are detectable in human populations. Extending this further, evidence exists for sex-specific effects on DNA methylation at the H19 ICR and IGF2 DMR2 in early second-trimester human fetal livers following in utero exposure to cigarette chemicals (241). Importantly, these sex-specific effects are associated with depleted levels of hepatic cobalt and vitamin B12, together with altered expression of DNMT1 and transcripts for several enzymes involved in 1C metabolism.

5.4. Transgenerational Epigenetic Inheritance

Overall, a compelling body of evidence from rodent studies supports the notion of transgenerational epigenetic and phenotypic inheritance following parental exposure to ECs (196, 249) (see the sidebar titled Mechanisms of Germline Epigenetic Transmission). A recent systematic review identified 43 EC-related articles reporting transgenerational epigenetic inheritance in rodents (196). No equivalent human or large animal studies were found in this search. Cited rodent studies involved a range of (mostly) single chemicals with known endocrine-disrupting effects including atrazine, BPA, phthalates, DDT, dioxins, and vinclozolin. F0 exposure to ECs occurred mainly during pregnancy, although a few studies considered F0 male exposure prior to mating, e.g., to methoxychlor (250). Most studies provided evidence of epigenetic transmission via the male germ line, reporting a range of chromatin modifications and altered populations of small ncRNAs in sperm and/or somatic cells. Some studies could confirm only intergenerational inheritance—i.e., F1 paternal [e.g., benzo(a)pyrene (251)] and F2 maternal [e.g., BPA (252)] transmission—whereas others found that differences in DNA methylation and the incidence of cryptorchidism had diminished by F4 [e.g., F0 pregnancy exposure to DEHP (253)].

To the best of our knowledge, there is currently no equivalent evidence of EC-induced transgenerational epigenetic inheritance in large outbred species such as humans and farm animals (196, 197, 254). Such effects were shown to attenuate DEHP-induced transgenerational epigenetic inheritance of TDS in FVB/N relative to C57BL/6J strains of mice (255). Genetic

MECHANISMS OF GERMLINE EPIGENETIC TRANSMISSION

A long-held belief was that due to the significant extent of germline-chromatin remodeling that takes place in mammals, epigenetic modifications acquired during the lifetime of an individual are erased and thus not passed on to the next generation. Such a contention adheres to the concepts of evolutionary biologist August Weismann, who distinguished the "immortal" germline from the "disposable" soma. Breaching the Weismann barrier requires a mechanism(s) by which acquired epimutations can escape germline erasure and be passed on to successive generations. Furthermore, to satisfy the definition of transgenerational epigenetic inheritance in mammals, this mechanism(s) must persist for at least two generations to confirm paternal transmission and three or more generations to confirm maternal transmission (242).

Research findings in the past 10–15 years are beginning to elucidate such mechanisms. Several have now been proposed for germline epigenetic inheritance incorporating direct replicative and indirect reconstructive modes of transmission (243, 244). Examples of the former include DNA methylation, particularly in the context of genomic imprinting, and histone modifications that escape germline erasure. A significant proportion of sperm-derived histones are retained following fertilization, and these could serve to replicate posttranslational modifications acquired during spermatogenesis (245). Among the putative mechanisms underpinning reconstructive transmission in mammals, the role of both maternally and paternally inherited noncoding RNAs has gained the greatest traction (246, 247). However, separating replicative from reconstructive modes of transmission is problematic, and emerging evidence indicates that they operate synergistically to direct epigenetic transgenerational inheritance, as witnessed in gestating female rats exposed to either the agricultural pesticide vinclozolin or the pesticide dichlorodiphenyltrichloroethane (248).

contributions to epigenetic and phenotypic outcomes have contributed to the controversy surrounding the significance of the few studies that have considered transgenerational epigenetic inheritance in outbred animal species and humans (199).

6. ASSESSING RISK OF ECs ON METABOLIC AND REPRODUCTIVE HEALTH

There are several major challenges to understanding the effects of real-life EC mixture exposure on public and environmental health. Core to this is the fact that the effects of ECs, when present within a mixture, do not necessarily reflect the sum of the effects of the individual chemicals/chemical types, which can be closely defined by laboratory studies. Indeed, when in a mixture, ECs may exhibit additive, synergistic, or even antagonistic effects, making prediction of effects highly complex (256). Many published laboratory studies document the effects of acute, high-dose EC exposure, whereas real-life EC exposure is typically chronic and not to overtly toxic levels of ECs. Extrapolation from such laboratory studies to predict what might be the effects of an EC mixture is also difficult, because EC dose-response profiles are often nonmonotonic (257). Finally, although some physiological effects may be mediated by the EC itself, they can also arise in response to in vivo metabolites of the EC, which may exhibit effects and work via different modes of action relative to their parent EC (1). For example, the plasticizer DEHP and its primary metabolite MEHP [mono-(2-ethylhexyl) phthalate] exhibit pro- and antiandrogenic activities, respectively (258).

When considering ECs as a mediator of DOHaD, an additional complication relates to the developmental time at which EC exposure occurs. The timing and coordination of normal fetal growth and development rely on complex signaling (endocrine and nonendocrine) that controls processes such as organogenesis, steroidogenesis, neuronal development, and metabolism. Therefore, the impacts of EC exposure will depend on the developmental stage at which it occurs.

For example, in the developing rat, exposure to an androgenic EC between embryonic day 15.5 and 19.5, which corresponds to weeks 8–14 of human gestation, would result in masculinization of reproductive tissues/structures (259), and blockade of normal androgen signaling is likely to result in male offspring with a TDS-like phenotype. In contrast, exposing rat pups to an androgenic EC, such as DBP, between embryonic day 13.5 and 15.5, i.e., before the masculinization window, does not affect the external appearance of the male genitalia but affects other aspects of reproductive function, including germ cell development (260). The exact mechanisms through which these developmental time-sensitive effects of ECs occur have not been characterized extensively but are likely to be the result of interactive effects of ECs, aberrant endocrine signaling, and the induction of molecular and/or epigenetic changes. Critically, however, real-life EC exposure is continual, although the balance of ECs that may have androgenic/antiandrogenic/estrogenic/antiestrogenic effects may vary across gestation and across other developmentally important periods. Finally, the effects of EC exposure may be affected by genetic factors, i.e., the genetic background and associated pharmacogenomic differences between study subjects/populations. This is a particular issue when considering laboratory studies that have used specific inbred strains or rats and mice in which inconsistent results may be strain dependent (257, 261–263) but may not represent the effects seen in a wild or outbred population.

7. CONCLUSION

Obesity, metabolic disease, alterations in pubertal timing, infertility, and CVD in humans are all linked to EC exposure. However, given the epidemiological basis of human studies, none have definitively established causation, and the mechanism(s) (including epigenetic) that underlie these pathologies remain to be elucidated fully. Our understanding of how ECs affect health is based largely on epidemiological studies in humans and wildlife (not considered in this article) combined with traditional toxicological (risk) assessments in animal models. Choice of appropriate animal models requires an understanding of species differences in lifespan, development, and physiology. The lack of genetic diversity in many laboratory species, although acceptable for controlled toxicological studies, renders them less translationally relevant with respect to effects of more general EC exposure. Future research should extend beyond single-chemical effects to prioritize real-life EC mixtures across multiple developmental stages and/or generations in outbred animal species. This approach would provide more representative platforms to investigate sexually dimorphic and individual effects of EC exposure. However, establishing the mechanistic basis for effects of EC mixtures will require a combined approach using in vivo and targeted in vitro (e.g., cell culture, organ-on-a-chip) models. Demonstrating causality and mode of action will facilitate the development of One Health strategies to mitigate the harmful developmental effects of EC exposure.

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