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Marked for Life: Epigenetic Effects of Endocrine Disrupting Chemicals

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Abstract
The presence of human-made chemical contaminants in the environment has increased rapidly during the past 70 years. Harmful effects of such contaminants were first reported in the late 1950s in wildlife and later in humans. These effects are predominantly induced by endocrine disrupting chemicals (EDCs), chemicals that mimic the actions of endogenous hormones and leave marks at several levels of organization in organisms, from physiological outcomes (phenotypes) to molecular alterations, including epigenetic modifications. Epigenetic mechanisms play pivotal roles in the developmental processes that contribute to determining adult phenotypes, through so-called epigenetic programming. While there is increasing evidence that EDC exposure during sensitive periods of development can perturb epigenetic programming, it is unclear whether these changes are truly predictive of adverse outcomes. Understanding the mechanistic links between EDC-induced epigenetic changes and phenotypic endpoints will be critical for providing improved regulatory tools to better protect the environment and human health from exposure to EDCs.

Keywords
endocrine disrupting chemicals, DNA methylation, histone modification, noncoding RNA, transgenerational epigenetic inheritance, chemical risk assessment
1. INTRODUCTION

More than 50 years ago the biologist Rachel Carson published a pioneering and eloquent collation of observations on the sentinel adverse reproductive effects of chemicals on wildlife. This led to the growth of public concern about the effects such chemicals might also be having on human populations, both at the start of life (including in the womb) and in later life, such as increased cancer risk. Some chemicals that in the 1950s seemed to have no apparent disbenefits were later discovered to travel extensively in the environment, persist for many years, accumulate in a range of species, and have adverse endocrine-related impacts on health. As public concern bloomed, intergovernmental initiatives were introduced to better screen and regulate such chemicals and thus improve environmental and public health protection. Our understanding of the modes and mechanisms underlying physiological responses to chemical exposures has developed a great deal, but it is only recently that a pivotal role for epigenetic mechanisms has begun to be unraveled. Here we review the current evidence for epigenetically mediated adverse outcomes resulting from exposure to endocrine disrupting chemicals (EDCs), exploring how epigenetic tools could be developed and utilized to better protect the environment and future generations.

2. ENDOCRINE DISRUPTION: MODES OF ACTION AND HEALTH EFFECTS

2.1. Wildlife and Human Exposure to EDCs: Growing Public Concern

The role of industrial chemical contaminants in causing environmental and human ill health has been observed since the Industrial Revolution. However, after World War II, there was a rapid proliferation in the use of highly persistent and fat-loving (lipophilic) chlorinated pesticides and industrial chemicals. In the late 1950s, observations were made of adverse impacts on wildlife. Species at the top of the food chain were particularly affected, for example, larger hunting birds such as eagles and hawks, whose reproductive success was disrupted by eggshell thinning. As
researchers started to analytically identify the unknown chemicals present in the affected parents and eggs, the growing body of evidence in wildlife stimulated the biologist Rachel Carson to publish her seminal and highly influential book *Silent Spring* in 1962 (1).

Public interest and concern steadily increased, along with an expanding body of wildlife evidence, which began to show that not only were many of these chemicals bioaccumulating further up the food chain but also that there was a long-range atmospheric transport effect. Many of the most persistent chemicals were significantly accumulating in remote polar regions, far from the original manufacturing, use, and waste treatment sites. Further examples continued to be observed in wildlife throughout the 1980s and 1990s. Some synthetic estrogen pharmaceuticals (such as the contraceptive pill) released into the aquatic environment dramatically altered sex ratios in fish in UK estuarine waters (2). Adverse effects were observed upon the reproductive health of alligators living in polluted lakes in the United States (3). Hermaphroditism was reported in arctic polar bears (4), vast distances from the origins of the persistent organic/EDC production and usage of persistent and organic pollutants (POPs) that act as EDCs. Such studies provided clear evidence that chemicals could (a) travel extensively around the globe (the so-called grasshopper effect), (b) be highly bioaccumulative, and (c) persist in the environment for a great many years. During the same time period, evidence in human epidemiological cohorts of endocrine disruption across multiple generations was accruing. This evidence began with the probable association of the synthetic estrogen diethylstilbestrol (DES) with a rare vaginal clear-cell adenocarcinoma in girls and young women who had been exposed in utero (5). DES was inappropriately prescribed to pregnant women between 1940 and 1971 to prevent miscarriage, premature labor, and related complications of pregnancy.

Thirty years after the publication of *Silent Spring*, the term endocrine disruptor (ED) was first coined at the Wingspread Conference in Wisconsin, United States, in 1991 (6). Shortly after, in 1996, Theo Colborn and colleagues (7) published *Our Stolen Future*. In the years that followed, a growing body of literature discussed the increasing incidence of breast cancer in women (8) as well as the decreased sperm counts and increasing incidence of testicular cancer in men (9). Together with adverse effects including birth defects, reproductive failures, and sexual abnormalities, this literature stimulated research into the chemical and molecular actions as well as the clinical and epidemiological effects in humans of a large variety of EDCs present in the environment (10). These effects extended beyond reproductive impairment and cancer to include cognitive impairment [polychlorinated biphenyls (PCBs)] and alterations in sex ratios (dioxins). Contaminant levels were increasingly being detected in humans, not just in hormonally active tissues, such that the chemical contamination of human breast milk became a very hot topic of discussion in the 1990s.

In response to public concern, some governmental endocrine relevant chemical screening programs were created, and in 1998, the intergovernmental Organisation for Economic Co-operation and Development (OECD) work program on ED testing and assessment was initiated. The first ED tests focused on the estrogen, androgen, thyroid, and steroidogenesis pathways. However, the array of modes and mechanisms of action that require test method development are expanding (a) to include other related endocrine pathways (11) and (b) to explore specifically how epigenetic modes and mechanisms of action could be included (12, 13), particularly with respect to temporal considerations through life and for subsequent generations.

With respect to the more lipophilic POPs, additional measures were initiated at the global level to reduce and eliminate the most persistent and toxic chemicals. These initiatives continue to respond to the changing environmental chemical milieu. In 2001, the UN Stockholm POPs treaty was signed (and came into force in 2004). This global treaty is intended to protect the environment and human health from chemicals that remain intact in the environment for long
periods, become widely distributed in the environment via the grasshopper effect, accumulate in the fatty tissue of wildlife and humans, and induce adverse outcomes. While the chemicals included in the POP list (such as PCBs, organochlorine pesticides, and brominated flame retardants) are being phased out or banned from further production, public health risks remain until they have been safely removed from the environment and carefully disposed of. Also, many of the listed POPs remain in production for use in less developed countries, despite being restricted or banned in the manufacturing country, and there are many examples for which phase out and cleanup targets or deadlines have not yet been met. Figure 1 provides a timeline of the events described in this section.

**Figure 1**
Timeline perspective: EDs, public concern, and intergovernmental body responses. Bold text signifies key global events (not related to publications), red text signifies major endocrine and epigenetic physiological adverse health effects in wildlife and humans, green text signifies new ED fields that are starting to be addressed internationally. Abbreviations: DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; ED, endocrine disruptor; OECD, Organisation for Economic Co-operation and Development; POP, persistent organic pollutant; WHO, World Health Organization.

| Year | Event |
|------|-------|
| 1929 | Industrial production of electrical conduction fluids: polychlorinated biphenyls for transformers, etc., begins |
| 1940s | Insecticidal properties of DDT discovered, industrial production begins |
| 1950s | Reproductive failure starts to be observed in birds of prey |
| 1962 | Silent Spring by Rachel Carson is published |
| 1962 | DES prescription starts |
| 1962 | Insecticidal properties of DDT discovered, industrial production begins |
| 1971 | Association between DES and vaginal adenocarcinoma of girls and young women exposed in utero |
| 1991 | Wingspread Conference: term endocrine disruptor is coined |
| 1996 | Our Stolen Future by T. Colburn et al. is published |
| 1998 | OECD Endocrine Disruptor Testing and Assessment work program established |
| 2001 | Stockholm POPs convention ratified (initiated in 2001) |
| 2002 | WHO report and definition of endocrine disruptor introduced |
| 2004 | Intergovernmental focus on estrogen, androgen, thyroid, and steroidogenesis pathways for endocrine disruptor toxicity testing |
| 2004 | Stockholm POPs convention ratified (initiated in 2001) |
| 2006 | Incorporation of epigenetics into endocrine disruptor testing? |
2.2. The Endocrine System: The Target of EDCs

Here we describe how the endocrine system functions in mammals, how it might be perturbed by EDCs, and what this means for human and environmental health outcomes.

2.2.1. The endocrine system. The body is composed of many millions of cells with different specialized functions, all of which need to work together as a whole and communicate with widely distributed cells and organs. To enable this, various forms of complementary intercellular communication exist between cells and organs. Nervous system communication is characterized by direct, rapid, and short-lived signals between individual cells. In contrast, the endocrine system allows for slower, more prolonged communication between large numbers of cells at many different sites in the body (14). During endocrine signaling, a group of secretory cells (i.e., a gland) secretes a potent chemical transmitter (a hormone) into the blood. The transmitter is then carried by the blood to the target cells where a response is elicited. The endocrine system contributes to a multifaceted signaling feedback system that regulates basal processes such as metabolism, growth, and reproduction. It remains underdeveloped in the growing fetus and infant and so is particularly vulnerable to toxic insults during early life. Since the early 2000s, it has become increasingly evident that epigenetic processes play a pivotal role in regulating many mammalian genes involved in the endocrine processes. Table 1 gives an overview of genes regulated by DNA methylation (an epigenetic mark; see Section 3), and the major endocrine pathways in which they are involved.

The major glands of the endocrine system are the pituitary, thyroid, parathyroid, pancreas, gastrointestinal tract, adrenal, reproductive (testes and ovaries), hypothalamus, and pineal. The latter two have a role in controlling the activities of many of the other endocrine glands.

Hormones are endogenously produced within the human body. Exogenous chemicals can, however, mimic endogenous hormones, sometimes to such an extent that the hormone pathway becomes perturbed beyond what is manageable by the processes responsible for maintaining a constant balanced internal environment (homeostasis). There are two major classes of endogenous hormones. Peptide hormones are composed mainly of amino acids and are initially made and stored as inactive molecules that can be cleaved when the active hormone is required (such as gastrin). Steroid hormones all have a specific structure, the cyclopentanoperhydrophenanthrene nucleus. This is the key designed to unlock the activity of the specific steroid hormone receptor (SHR) to which such hormones bind. Our review focuses on the steroid hormone class because interference with this hormone class constitutes both the main and the most-studied route by which chemical hormone mimics can disrupt the endocrine system. Unlike the water-soluble peptide hormones, steroid hormones are lipophilic and so move easily through cell membranes to exert their effects. In combination with each other, they contribute to the regulation of broad aspects of growth, development, and adult organ physiology.

2.2.2. Molecular mechanisms of steroid hormone action

2.2.2.1. The nuclear receptor family. There are many different steroid hormones and therefore many different SHRs, all belonging to the large family of nuclear receptors (NRs). While the SHR family is specific to steroid hormone signaling, the NR family also encompasses receptors, such as the pregnane X receptor (PXR), constitutive androstan receptor (CAR), and peroxisome proliferator activated receptors (PPARs) that play a major role in clearing up excess endogenous hormones (including steroid hormones) and exogenous substances (xenobiotics), such as hormone
Table 1  Examples of mammalian endocrine genes regulated by DNA methylation (with endocrine pathway information from Reference 119), including primary endocrine mode of action pathways

| Gene name       | Gene symbol | Endocrine pathway and mode of action                                                                 |
|-----------------|-------------|-------------------------------------------------------------------------------------------------------|
| P450scs         | CYP11A1     | Steroidogenic pathway: steroidogenic cleavage enzyme                                                  |
| 3β-hydroxysteroid dehydrogenase | HSD3B1/2 | Steroidogenic pathway: steroidogenic cleavage enzyme                                                  |
| 17α-hydroxylase | CYP17A1     | Steroidogenic pathway: steroidogenic progesterone metabolism to 17α-hydroxylase                      |
| 17β-hydroxylase | HSD17B3     | Steroidogenic pathway: steroidogenic cleavage enzyme androgen synthesis                              |
| Vitamin D synthesis | CYP27A1/B1 | Calcium (and bone) homeostasis                                                                      |
| Androgen receptor | AR         | Androgenic                                                                                           |
| Estrogen receptor 1 | ESR1     | Estrogenic, ovulation                                                                               |
| Estrogen receptor 2 | ESR2     | Estrogenic                                                                                           |
| Progesterone receptor | PGR      | Steroidogenic pathway: steroidogenic intermediate for estrogen and androgens, ovulation             |
| Glucocorticoid receptor | NR3C1   | Glucocorticoid: cortisol and glucose metabolism, lipolysis, and mobilization of fatty acids         |
| Mineralocorticoid receptor | NR3C2  | Renin-angiotensin system, aldosterone, and antiuretic hormone: blood volume and blood pressure       |
| Retinoic acid receptor α | RARA     | Retinoid metabolism                                                                                 |
| Retinoic acid receptor β | RARB     | Retinoid metabolism                                                                                 |
| Somatostatin   | SST        | Thyroid: inhibits thyroid-stimulating hormone                                                        |
| Insulin        | INS        | Glucose metabolism                                                                                  |
| Leptin         | LEP/OB     | Appetite control                                                                                    |
| Oxytocin receptor | OXTR     | Lactation: positive-feedback loop                                                                    |
| Follicle-stimulating hormone receptor | FSHR | Ova maturation and spermatogenesis process initiation: under hypothalamic gonadotropin control |
| Thyroid-stimulating hormone receptor | TSHR | Thyroid: long negative-feedback loop                                                                 |
| Insulin-like growth factor receptors | IGF1R/IGF2R | Glucose metabolism                                                                                 |

**Gene expression**: the process of producing a functional product from a gene, involving the transcription of DNA into RNA [such as messenger RNA (mRNA) that is subsequently translated into protein, or noncoding RNA].

Medication or pollutants. Receptors are proteins, synthesized by the cell from DNA in the same way as other proteins. Thus every cell has the ability to produce all receptors, but variations in gene expression mean that individual cells or tissues do not generally synthesize receptors for more than three or four hormones (14). This is considered to underlie the selectivity of hormones on their target tissues. Activation of NRs regulates the majority of gene expression involved in fat, glucose, cholesterol, bile acid, endogenous hormone, and xenobiotic metabolism.

The NR family has common structural features. The central highly conserved DNA binding domain targets the NR to specific DNA sequences, termed hormone response elements (HREs). The ligand binding domain (LBD) directly interacts with the hormone or chemical mimic. Embedded within this LBD is a hormone-dependent transcriptional activation domain. The LBD acts as a molecular switch, recruiting coactivator proteins and activating the transcription of target genes when flipped into an active conformation by hormone binding. This is known as the “lock and key” hypothesis, where a hormone must have a configuration specific to that of the LBD to activate the NR. Some hormones or chemical mimics are not quite the appropriate shape, but they can still occupy and interact with the NR. Such interactions can affect the ability of the NR to
produce a response, resulting in partial activation (agonism), repression (antagonism), or complete antagonism by blocking access to the LBD.

The long accepted theory of steroid hormone binding suggests that, in the absence of the hormone, each NR is associated with a chaperone complex (containing proteins that protect and aid the receptor, such as heat shock proteins) (15). Binding of the steroid hormone to the NR causes a conformational change. This molecular switch results in the removal of the chaperone complex and allows the NRs to dimerize. A homodimer is formed from two identical molecules, a process called homodimerization. A heterodimer is formed from two different molecules, a process called heterodimerization. Binding of the NR dimers to HREs within regulatory DNA regions leads to the recruitment of coregulators that promote activation (coactivator) or repression (corepressor) of target gene transcription (15–17). Figure 2 illustrates the basic NR-mediated mechanism of gene activation.

2.2.2.2. The oestrogen, progesterone, and androgen receptors. Estrogen receptors (ERs) are the most studied of all the NRs and may be the ancestral origin for the full complement of present-day mammalian SHRs (19), many of which are shown in Table 1. There are several subtypes, each encoded by a separate gene. The two main subtypes first identified were ER alpha (ERα) (20) and ER beta (ERβ) (21) along with their isoforms. Although ERα and ERβ evolved in parallel, the ancient duplication was to facilitate unique roles in vertebrate physiology and reproduction. After the advent of jawed vertebrates 450 million years ago, the progesterone receptor evolved. This was followed by the androgen receptor (AR), which also exists in different isoforms. Specific regulation of physiological processes by androgens and corticoids emerged after these events.
Both ER and AR variants occur not only in the nucleus, but also in the cytoplasm of the cell. Owing to the relatively early identification of (a) the ERα, and (b) links between the insecticide dichlorodiphenyltrichloroethane (DDT) and breast cancer in women and androgen-related reproductive disorders in men, ERs and ARs have received the most research and chemical regulatory attention with respect to EDCs. Although their principal mode of action is direct transcriptional regulation, they can also regulate gene expression indirectly without binding to DNA. ERs, ARs, and progesterone receptors homodimerize upon activation by their ligand(s), whereas many other NRs heterodimerize to activate specific pathways. In reality, the modes and mechanisms of action are not so simple. SHRs and other NRs all have differing ligand binding and activation formats; they also have both similar and different chaperone proteins and coregulators to facilitate or suppress selectivity.

2.2.2.3. The key role of endocrine metabolism in hormonal homeostasis. SHRs, other NRs, and chaperone proteins also mediate hormonal homeostasis by the coordinated release and degradation of bioactive hormones. Steroid hormones, their metabolites, ingested plant and animal steroids, and bioactive xenobiotic compounds are primarily metabolized by cytochrome P450 (P450) enzymatic reduction and oxidation in the liver. Many P450s have broad substrate activity and appear to be integrated into a coordinated metabolic pathway. Although some of the receptors inducing P450s (e.g., PPARs) are ligand specific, others (e.g., PXR) have broader specificity and low ligand affinity (Table 2). In this way, they can monitor aggregate levels of substrates to trigger production of metabolizing enzymes, thereby mounting a defense against toxic compounds in the diet. P450 induction by EDCs and other xenobiotics may therefore lead to alterations of endogenous regulatory pathways, with associated adverse physiological consequences.

Several steroidogenic pathway cleavage enzymes such as P450ccc and aromatase (CYP17A1, CYP19A1) are also involved in the derivation of the active forms of progesterone, testosterone, and estradiol from cholesterol. The steroidogenic acute regulatory protein (StAR), which regulates cholesterol transfer within the mitochondria and thus is the rate-limiting step in the production of steroid hormones, is also an important steroidogenic mechanism that can be modulated. Other relevant but receptor-independent steroid enzyme systems include sulfotransferases and N-acetyltransferase 1.

2.3. Endocrine Disruption: Definition and Health Effects

Many substances released into the environment through human activity can interfere with the endocrine or hormone systems of animals and humans. Endocrine-active substances occur both naturally and in a variety of chemical classes such as synthetic drugs, pesticides, chemicals used in industry and consumer products, and industrial by-products and pollutants, including some metals. The term “endocrine active substance” (EAS) is used to describe any chemical that can interact directly or indirectly with the endocrine system, and subsequently result in an effect on the endocrine system, target organs and tissues. Whether the effect is adverse (“disruptive”) or not will depend on the type of effect, the dose and the background physiological situation (22, p. 6). The body may be able to restore its balance, particularly if exposure to the source is very limited or if the substance is water soluble and rapidly metabolized and excreted, which is generally the case for plant-derived estrogens (phytoestrogens). Similarly, with pharmaceuticals such as the contraceptive pill, the human body can restore its balance following cessation of exposure in adulthood. For young girls, however, untimely exposure can lead to precocious puberty. Such disruption is incorporated in the term EDC when a link with adversity is displayed: “An endocrine disrupter is an...
Table 2  Nuclear receptors inducing major CYP enzyme classes

| Receptor                                      | Heterodimerization partner | Prototype ligands                                                                 | Enzyme(s)   | Main purpose and organ of activity | Major cross talk with |
|-----------------------------------------------|----------------------------|-----------------------------------------------------------------------------------|-------------|------------------------------------|-----------------------|
| Aryl hydrocarbon receptor (AhR)               | Aryl hydrocarbon receptor nuclear translocator (ARNT) | Dioxins, PAHs (e.g., in tobacco smoke), benzo-a-pyrene                             | CYP1A, CYP1B| Xenobiotic metabolism, Liver       | ER                    |
| Pregnane X receptor (PXR)                     | Retinoid X receptor (RXR)  | Rifampicin, steroids, drugs                                                        | CYP3A       | Steroids, xenobiotic metabolism, Liver | CAR                   |
| Constitutive androstane receptor (CAR)       | RXR                        | 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, TCPOBOP                              | CYP2B       | Steroids, xenobiotic metabolism, Liver | PXR                   |
| Peroxisome proliferator receptor α (PPARα)   | RXR                        | Long-chain polyunsaturated fatty acids, phthalate esters, e.g., bis(2-ethylhexyl) phthalate (DEHP) | CYP4A       | Fatty acid metabolism, Liver       | PXR                   |
| Vitamin D receptor (VDR)                     | RXR                        | Vitamin D: calcitriol                                                             | CYP27A1/B1: indirectly control CYP3A4 expression through transcriptional regulation | Calcium (and bone) homeostasis, Thyroid (initial synthesis), bone, and kidney | Parathyroid hormone |
| Retinoic acid receptor (RAR)                 | RXR                        | Retinoic acid (RA)                                                               | CYP26A1 requires the activity of RARβ2, which can be further activated by functional RARα in response to RA | Retinoid metabolism, Liver | PPAR                   |

Abbreviations: ALDH1A2, aldehyde dehydrogenase 1 family member A2 gene; PAHs, polycyclic aromatic hydrocarbons.
Noncoding RNAs (ncRNAs): RNAs transcribed from nonprotein coding DNA, which mediate gene expression via chromosome remodeling and transcriptional or posttranscriptional regulation

Histone modification and DNA methylation: addition or removal of a wide range of chemical moieties to histones or addition of methyl groups to DNA, all of which regulate gene expression by altering the interaction of DNA with DNA binding proteins involved in transcription (such as transcription factors and RNA polymerase)

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” (23, p. 1).

EDCs are also distinguished from classical toxicants such as cancer-causing substances (carcinogens), neurotoxicants, and heavy metals because they can interfere with normal blood hormone levels or the subsequent action of those hormones and induce outcomes at levels far lower than those of traditional concern to toxicologists. In general, EDCs are lipophilic and, at least in part, structurally similar to the endogenous hormone ligand they mimic. At times, high doses shut off effects that occur at low levels, and at others, low and intermediate doses produce greater effects than those observed at high levels (so-called non-monotonic dose responses). This is because the same EDC can have multiple modes of action and/or act as both an agonist and antagonist, all depending on concentration. EDC-induced effects can influence and disrupt the hormonal regulation and hormonal imprinting of normal cell proliferation, differentiation, and metabolism throughout life.

Furthermore, human populations are exposed to complex mixtures of environmental and endogenous agents, which may act together or modulate one another to produce biological effects. Endocrine disruption can involve any hormonal system and may be affected by normal physiological states [such as menstruation and menopause in women, andropause in men, and puberty and age-related reduction in growth hormone (somatopause) and hormones from the adrenal glands (adrenopause) in both sexes], diet, stress, and other lifestyle factors. Thus, as described above, an EDC acts by upsetting the multifaceted endocrine system to such a level that normal homeostatic balance is not able to restore itself quickly enough, leading to developmental malformations, interference with reproduction, increased cancer risk, and/or disturbances in immune or nervous system function.

If EDC-induced effects occur during critical and highly sensitive windows of development, they can lead to profound and lasting effects that may be transmitted to future generations. Fetal development malformations, particularly in reproductive organs, for example, undescended testicle(s) (cryptorchidism), shortened distance between the anus and genitals (anogenital distance), and abnormal location of urinary opening (hypospadias), are developmentally irreversible and can be corrected only by surgery. With respect to nervous system function, cognitive development may be impaired, leading to long-term IQ deficits and attention deficit disorders. Endocrine processes and tissues throughout the body may also be specifically affected and in addition to the above listed effects include associations with obesity, diabetes, and stress. Such adverse effects may be seen at individual and cohort levels as well as population levels, and skewed sex ratios have been reported for both wildlife and human populations.

3. POTENTIAL EPIGENETIC EFFECTS OF ENDOCRINE DISRUPTING CHEMICALS

3.1. Brief Overview of Epigenetic Mechanisms and Their Functions

For the purposes of this review, epigenetic changes are defined simply as any long-term change in gene expression that persists even when the initial trigger is long gone and does not involve a change in gene sequence or structure (24). They include histone modification, DNA methylation, and noncoding RNAs (ncRNAs). The epigenome represents the complete collection of epigenetic modifications within a cell at any given time, whereas the genome encompasses the entire genetic material within a cell at any given time.

Histone modification and DNA methylation both involve the removal and/or addition of specific moieties to particular components of chromatin, i.e., the protein-DNA complex that enables packaging of DNA into the nucleus (25). Histones are the highly conserved proteins around which
DNA winds to form the main structural units of chromatin (nucleosomes). These histones (predominantly the tails) can undergo multiple enzymatic modifications, such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation, giving rise to the so-called histone code (26, 27). These modifications are added and removed by a wide range of histone-modifying enzymes, including histone acetyl transferases, which add acetyl groups; histone deacetylases (HDACs), which remove acetyl groups; histone methyl transferases, which add methyl groups; and histone demethylases, which remove methyl groups. Likewise, functional methyl groups can be enzymatically added directly to DNA, primarily to the C5 of cytosine within cytosine-phosphate-guanine dinucleotides (CpGs) to produce 5-methylcytosine. CpGs are nonrandomly distributed throughout the genome, forming distinct CpG-rich regions known as CpG islands, which are predominantly associated with regulatory elements such as promoters (28, 29). Methyl groups are added to DNA by DNA methyltransferases (DNMTs) and removed through a combined process of oxidation to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine, and 5-carboxylcytosine by the ten-eleven translocation methylcytosine dioxygenase (TET) enzymes, followed by repair by thymine DNA glycosylase (TDG) together with the base excision repair machinery (30). Both histone modification and DNA methylation alter the interaction between DNA and DNA binding proteins (such as transcription factors and RNA polymerases) so that the overall combined effect of local modifications controls gene expression. In general, in promoter regions, histone deacetylation/methylation and DNA methylation are associated with transcriptional repression, whereas histone acetylation/demethylation and lack of DNA methylation are associated with transcriptional activation.

ncRNAs are produced from the transcription of nonprotein coding DNA and are broadly classified according to length: long noncoding RNAs (lncRNAs) (>200 nt) and short ncRNAs (sncRNAs) (<200 nt, typically 19–30 nt), which include microRNAs (miRNAs), piwi-interacting RNAs, and endogenous short interfering RNAs. Whereas lncRNAs mediate gene expression in a variety of ways, such as chromosome remodeling and transcriptional or posttranscriptional regulation, sncRNAs predominantly negatively regulate gene expression at the posttranscriptional level (31, 32).

These epigenetic processes do not act independently; they interact to form complex regulatory epigenetic networks that function to enable adaptation to environmental change (phenotypic plasticity) and help maintain homeostasis. Therefore, all the machinery involved in these pathways, including the DNA and RNA binding and modifying proteins (epigenetic machinery), are epigenetically important. As such, epigenetic mechanisms play critical roles during normal development and in the maintenance of functional tissue-specific gene expression. An individual comprises multiple specialized cell types capable of performing a wide range of diverse functions. These different cell types contain identical DNA. Thus, it is the differential epigenetic mechanisms within a specific cell type that determine which functionally relevant genes are switched on/off during cellular differentiation.

To enable proper cell differentiation, epigenetic patterns are reprogrammed twice during early development (13). The first reprogramming event immediately following fertilization promotes dedifferentiation so the resulting fertilized egg (zygote) becomes capable of producing all the different cell types required to form a new individual. This involves genome-wide DNA demethylation, histone modification, and ncRNA expression to “reset” the zygote genome. Epigenetic programming mediated by DNA methylation, histone modification, and ncRNAs then guides the zygote through embryo formation and development (embryogenesis) and ultimately into a mature adult. The second reprogramming event involves only primordial germ cells (PGCs), which ultimately form the mature germ cells (gametes, either egg or sperm). For future generations to reproduce successfully, the epigenetic marks on imprinted genes within PGCs must be
removed and reestablished according to the sex of the embryo. Imprinted genes carry a parent-of-origin epigenetic mark, ensuring expression from a single parental allele and the correct dosage of maternally and paternally derived gene products. Either the maternal or the paternal allele is epigenetically silenced. In most cells, these epigenetic marks are maintained throughout life. However, in PGCs, they are reset according to the sex of the embryo. Thus, PGCs undergo a second round of genome-wide demethylation and changes in histone modification and ncRNA expression.

As with the endocrine system, complex multifaceted epigenetic networks help maintain cellular homeostasis. They are also open to environmental perturbation, for example, via the action of EDCs (see Section 3.3). When environmentally induced epigenetic changes exceed a cell’s capacity to maintain homeostasis, epigenetic toxicity can occur. It is easy to envisage how epigenetic changes during early development could be deleterious to future health. However, the same epigenetic processes also drive cell proliferation, differentiation, function, and adaptation in adulthood. Thus, epigenetic perturbation can occur throughout an individual’s life, resulting in abnormal cell growth or function and, ultimately, disease development. For example, adult-onset diseases such as cancer and diabetes as well as neurological, renal, reproductive, cardiac, and respiratory conditions have all been associated with abnormal epigenetic changes (33–37). In addition, epigenetic processes are involved in the commencement of puberty, which entails changes that may also be associated with an increased risk of some adult onset diseases (38).

The increasing evidence for specific EDC-induced epigenetic effects and the resultant phenotypic consequences in wildlife, humans, and experimental model systems are considered in turn. To provide a nonbiased review of the current evidence, we searched the PubMed database using the search terms provided in Supplemental Table 1. The literature is vast, with many thousands of published studies. Therefore, only studies that measured both adverse phenotypes(s) and region-specific epigenetic change(s) (not general changes across the genome, nor those involving alterations to epigenetic machinery, such as modifying enzymes) following exposure to EDC(s) were included. In addition, because epigenetic marks are (as per our definition) long-lasting, we excluded studies that described only short-term events such as histone modifications directly linked to transcriptional activation. Thus, the final study lists provide the most relevant, and likely epigenetically mediated, EDC-induced pathophysiological outcomes.

3.2. Epigenetic Effects of EDCs in Natural Cohorts and Experimental Systems

Here we describe the current observational evidence base and mechanistic understanding of EDC-induced epigenetic effects within natural (wildlife and human) cohorts and experimental systems, exploring the actual and potential implications for current and subsequent generations.

3.2.1. Wildlife. As discussed above, the harmful effects of EDCs were first identified in wild populations. There are now many examples of EDC-induced phenotypes in wildlife. However, only two studies identified in PubMed demonstrated both altered health outcome(s) and specific epigenetic change(s) (DNA methylation) following exposure to EDC(s). The first reported increased intragenic DNA methylation of the follicle stimulating hormone receptor (Fshr) gene within the gonad tissue of juvenile female European eels (Anguilla anguilla) sampled from highly polluted compared with lightly polluted French waters, which correlated with increased levels of gonadal POPs and metals, decreased Fshr mRNA, and reduced gonad development in the eels from the highly polluted environment (39). The second measured reproductive impairments in 75–85 differentially methylated DNA regions in the red blood cells sampled from adult male American alligators (Alligator mississippiensis) living in POP and metal contaminated lakes (40). Genes
associated with the differentially methylated DNA regions were within pathways of endocrine relevance. Neither of these studies further investigated the potential epigenetic mechanism(s) identified. Although a third study demonstrated specific DNA methylation changes within the hepatic tumors of Dab flatfish (*Limanda limanda*) sampled from UK waters, it did not attempt to identify the potential contaminants responsible for the unusually high prevalence of liver tumors in these fish (41). Two other studies demonstrated global changes in DNA methylation within wildlife cohorts (42, 43), and additional studies investigated potential epigenetic mechanisms involved in EDC-induced toxicity in ecologically relevant species within an experimental laboratory-based setting (reviewed in 44–47) (also see Section 3.2.3). Thus, while there is only a small amount of direct mechanistic evidence for the existence of EDC-induced epigenetic toxicity in wildlife, interest in epigenetic ecotoxicology is growing and further investigations are ongoing.

### 3.2.2. Epigenetic effects of EDCs in humans.

Again, as discussed above, following evidence that EDCs can induce harmful effects in wildlife, concern over similar adverse outcomes in EDC-exposed human populations began to increase. Since the publication of the epidemiological study linking in utero exposure to DES with vaginal adenocarcinoma in girls and young women (5), evidence for EDC-induced phenotypic effects in humans has been growing. Many epidemiological studies, particularly from the 1990s onward, have now demonstrated an association between specific EDC(s) and adverse health outcome(s). Mechanistically linking exposures to harmful effects in human cohorts, however, is challenging for several reasons: For example, (a) access to the tissue of interest is often limited, and surrogate tissue (often blood) has to be used; (b) developmental exposure is difficult to assess and requires long-term longitudinal cohorts; (c) 80–90% of the human population is exposed to a mixture of EDCs on a daily basis, so there are no negative controls; (d) multiple potential confounders can be present and must be controlled; and (e) epidemiological associations do not demonstrate causality. Nevertheless, we identified in PubMed, 37 studies that associated adverse phenotype(s) with specific epigenetic change(s) (mainly DNA methylation but also miRNAs) and exposure to EDC(s) during various stages of development (updated from 13) (Table 3).

These studies are largely focused on cigarette smoke exposure, with some examples of effects induced by another lifestyle factor (alcohol) and by specific environmental chemicals. The studies vary in terms of size and nature of cohort, sampling time and tissue type, use of relevant controls, correlation of changes with gene expression at the mRNA and/or protein level, method of statistical analysis, validation in additional and/or independent cohorts, and further investigation of associations in a relevant in vivo and/or in vitro system. Only 8 of the 37 studies performed further in vivo and/or in vitro mechanistic investigations, which included exposures to cigarette smoke and environmental chemicals such as benzo[a]pyrene, benzophenone, bisphenol A (BPA), and polycyclic aryl hydrocarbons (PAH). These eight studies, four of which directly sampled the target tissue or validated the same change in the blood and target tissue of an appropriate model, provide the most comprehensive mechanistic evidence for the existence of EDC-induced epigenetic toxicity in human populations.

### 3.2.3. Epigenetic effects of EDCs in experimental systems.

The first indications that hormones could regulate epigenetic marks, and thus the notion that perturbation of hormonal signaling could alter the epigenome, were noted more than 30 years ago. In 1984, investigators reported that estrogen not only transcriptionally activated chicken vitellogenin but also actively demethylated the *vitellogenin* gene around the ER binding site (48). Almost 20 years later in 2001, similar effects were observed for glucocorticoids at the rat tyrosin aminotransferase gene (49). Yet, conducted during the era when research focused on the genome, these studies received little
| Environmental exposure | Human cohort | Measurements (stage of development and type) | Correlation with gene expression | Reference |
|------------------------|-------------|---------------------------------------------|----------------------------------|------------|
| **Chemicals**          |             |                                             |                                  |            |
| Benzo[a]pyrene         | Zhongshan Hospital of Xiamen University patients ($n = 189$, mean age 59 years) | Lifetime Serum | Adulthood ↑ Risk of HCC | Adulthood Serum ↑ GSTP promoter DNA methylation | Yes (in an in vitro model) | 12\textsuperscript{b,c} |
| Benzenophene 3         | NJMU birth cohort ($n = 423$ mother-child pairs) | In utero Maternal urine | Infancy (mean age 3 months) ↑ Risk of HSCR | Infancy (mean age 3 month) Colon tissue ($n = 204$) ↑ miR-218 | Yes | 12\textsuperscript{b,c} |
| Bisphenol A            | CCCEH cohort ($n = 198$, age 3–5 years) | In utero Maternal urine (GW24–40) | Childhood Behavioral abnormalities | Neonatal Cord blood ($n = 81$) ↑ BDNF promoter DNA methylation | Yes (in an in vivo model) | 12\textsuperscript{b,c} |
| Cadmium                | Three villages in south China: highly, moderately, and not polluted ($n = 81$, mean age 53 years) | Lifetime (48–59 years) Urine and blood | Adulthood (48–59 years) Renal dysfunction | Adulthood (48–59 years) Blood ↑ Intragenic RASAL1 DNA methylation | NA | 125 |
| Dichlorodiphenyl- dichloroethylene | Menorca cohort within INMA project ($n = 122$, age 4 and 6 years) | Maternal prenatal Cord blood | Childhood (6 years) ↑ Risk of persistent wheezing | Childhood (4 years) Blood ↓ Intragenic ALOX12 DNA methylation | NA | 126 |
| Perfluorooctanoic acid | Fluorochemical plant workers ($n = 41$, mean age 27 years) | Adulthood Serum | Adulthood Serum ↓ Serum HDL cholesterol | Adulthood Serum ↑ miR-26b and mi199a-3p | NA | 127 |
| Phthalates             | CEAS cohort ($n = 256$ mother-child pairs, age 3 years) | Lifetime Urine | Childhood Asthma | Childhood Blood (WBCs) ↓ TNFα promoter DNA methylation | Yes | 128 |

*(Continued)*
| Environmental exposure | Human cohort | Exposure | Adverse phenotype | Epigenetic change | Correlation with gene expression | Reference |
|------------------------|-------------|----------|-------------------|------------------|---------------------------------|-----------|
| PAHs                   | CCCEH cohort (n = 56, age 0–5 years) | In utero (GW 29–40) Air monitoring | Childhood (≤ 5 years) Asthma | Neonatal Cord blood (WBCs) ↑ACSL3 promoter DNA methylation | Yes | 129b,c |
| PAHs                   | Coke-oven workers plus controls (n = 128, mean age 42 years) | Adulthood Urine | Adulthood PBL chromosomal aberrations | Adulthood Blood (PBLs) ↑Intragenic p19INK4a DNA methylation | Yes (in an in vitro model) | 130b,c |
| PAHs                   | LIBCSP study (n = 765–851, age 20–98 years) | Adulthood Various routes of exposure (I/Q) | Adulthood ↑Prevalence in breast tumors | Adulthood Tumor tissue ↑CDH1, HIN1, P4RPβ, and TWIST promoter DNA methylation ↓BRCA1, CCND2, DAPK, and ESR1 promoter DNA methylation | NA | 131b |
| Lifestyle              | Alcohol     | Adulthood (30–75 years) Dietary intake (I/Q) | Adulthood (30–75 years) ↑Risk of CRC | Adulthood (30–75 years) Tumor tissue ↓IGF2 DMR-0 DNA methylation | NA | 132b |
| Smoking                | ELEMENT cohort (n = 80 mother-child pairs, 80% were 20–35 years) | Maternal preconception Smoking status (I/Q) | Neonatal ↑Gestational age | In utero Cervical cells (GW16–19) ↑PTGER2 promoter DNA methylation | NA | 133b |
| Smoking                | LINA cohort (n = 346 mother-child pairs) | In utero Smoking status (I/Q) | Childhood (age 1 year) Allergic disease (I/Q and serum IgE) | Neonatal Cord blood ↓FOXP3 promoter DNA methylation | NA | 134 |

(Continued)
| Environmental exposure | Human cohort | Exposure | Adverse phenotype | Epigenetic change | Correlation with gene expression | Reference |
|------------------------|-------------|----------|------------------|------------------|---------------------------------|-----------|
| Smoking                | University Hospital of North Staffordshire antenatal patients (n = 129 mother-child pairs) | In utero Smoking status (I/Q) | Neonatal ↑ Risk of birth weight < 50th percentile | Neonatal Cord blood ↓ GSTM5 and MAP2K3 DNA methylation ↑ APOB DNA methylation | NA | 135 |
| Smoking                | TBPS cohort (n = 150 mother-child pairs) | In utero Smoking status (I/Q and cord blood cotinine) | Childhood (2 years) Atopic dermatitis | Neonatal Cord blood (WBCs) ↓ TSLP promoter DNA methylation | Yes | 136 |
| Smoking                | BAM BAM study (n = 100 mother-child pairs) | In utero + neonatal Smoking status (Maternal and infant saliva or meconium cotinine) | Neonatal (over first month) Neurobehavioral abnormalities (HPA dysregulation) | In utero + neonatal Placental tissue (n = 45) ↓ NR3C1 promoter DNA methylation | NA | 137 |
| Smoking                | GECKO Drenthe birth cohort (n = 255 mother-child pairs) | In utero Smoking status (Maternal medical records) | Neonatal ↓ Birth weight | Neonatal Cord blood ↓ Intragenic GFI1 DNA methylation | NA | 138 |
| Smoking                | Saguenay youth study of adolescent sibling pairs (n = 956, mean age 15 years) | In utero Smoking status (I/Q) | Adolescence ↑ Fat intake (I/Q) | Adolescence Blood (PBLs) (n = 132) ↓ Intragenic OPRM1 DNA methylation | NA | 139 |
| Smoking                | Subsample of INMA project (n = 179 mother-child pairs) | In utero Smoking status (I/Q and maternal urine cotinine GW12 and 32) | Neonatal ↓ Birth weight | Neonatal Placental tissue ↑ DNA methylation of region between LINCO0086 and LEKRI ↑ DNA methylation of TRIO | NA | 140b |
| Environmental exposure | Human cohort | Exposure | Adverse phenotype | Epigenetic change | Correlation with gene expression | Reference |
|------------------------|-------------|----------|-------------------|-------------------|---------------------------------|-----------|
| Smoking                | Kanagawa Cardiovascular & Respiratory Center lung patients ($n = 87$, mean age 65 years) | Adulthood (37–84 years) Smoking status (I/Q) | Adulthood (37–84 years) Poor prognosis (LADC) | Adulthood (37–84 years) Tumor tissue ↑ MGMT promoter DNA methylation | Yes (protein level) | 141b |
| Smoking                | Yamagata University Hospital patients ($n = 51$ tumor-normal pairs, mean age 68 years) | Adulthood Smoking status (I/Q) | Adulthood ↑ Prevalence in NSCLC | Adulthood Lung tissue ↑ p16 promoter DNA methylation | Yes (protein level) | 142b |
| Smoking                | Massachusetts General Hospital patients ($n = 178$, median age 69 years) | Adolescence/adulthood Smoking status (I/Q) | Adulthood ↑ Prevalence in LADC tumors | Adulthood Tumor tissue ↑ RASSF1A promoter DNA methylation | NA | 143b |
| Smoking                | New Hampshire State Cancer Registry ($n = 331$, age 25–74 years) | Adulthood Smoking status (I/Q) | Adulthood ↓ Bladder cancer survival | Adulthood Tumor tissue ↑ CDKN2A, RASSF1A, APC, DAPK, PYCARD, LAMC2, PRSS3, RARB CDH13, and MGMT promoter DNA methylation | NA | 144b |
| Smoking                | Taipei & Taichung Veterans General Hospitals lung patients ($n = 124$, 72 % > 65 years) | Adulthood Smoking status (medical records) | Adulthood Poor prognosis (lung cancer) | Adulthood Tumor tissue ($n = 70$) ↑ DNMT1 protein level and nuclear localization (which led to ↑ FHIT, p16INK4a, and RAR promoter DNA methylation in vitro) | Yes (in an in vivo and in vitro model) | 145bc |
| Environmental exposure | Human cohort | Exposure | Adverse phenotype | Epigenetic change | Correlation with gene expression | Reference |
|-------------------------|-------------|----------|-------------------|-------------------|----------------------------------|-----------|
| Smoking                 | NYU Lung Cancer Biomarker Center patients (n = 80, mean age 53 years) | Adulthood (34–83 years) Smoking status (I/Q) | Adulthood (34–83 years) ↑ Prevalence in lung cancer | Adulthood (34–83 years) Blood ↑ KIF1A, DCC, RARB, and NISCH promoter DNA methylation | NA | 146 |
| Smoking                 | Ghent University Hospital patients (n = 32, mean age 57 years) | Adulthood Smoking status (I/Q) | Adulthood ↑ Prevalence in COPD | Adulthood Sputum ↓ let-7c and miR-125b | Yes | 147 |
| Smoking                 | Huai’an county patients (n = 120, mean age 62 years) | Adulthood Smoking status (I/Q) | Adulthood ↑ Risk of esophageal cancer | Adulthood Plasma ↓ miR-155 | NA | 148 |
| Smoking                 | NYU Lung Cancer Biomarker Center patients (n = 118, mean age 55 years) | Adulthood (34–85 years) Smoking status (I/Q) | Adulthood (34–85 years) ↑ Prevalence in lung cancer | Adulthood (34–85 years) Plasma ↑ NISCH promoter DNA methylation | NA | 149bc |
| Smoking                 | Subset of EPIC-Turin cohort (n = 374, median age 50 years) | Adulthood (34–65 years) Smoking status (I/Q) | Adulthood (34–65 years) ↑ Risk of breast cancer | Adulthood (34–65 years) Blood (WBCs, 2–9 years prior to diagnosis) ↓ DNA methylation of region 2q37.1 | NA | 150 |
| Smoking                 | Oncoradiology Center of the Markusovszky County Hospital patients (n = 48, mean age 62 years) | Adulthood Smoking status (I/Q) | Adulthood ↑ Prevalence in gastric cancer | Adulthood Gastric tissue ↑ miR-21 | NA | 151b |
| Smoking                 | National Cancer Center Hospital patients (n = 139 paired tumor-normal tissue samples, mean age 60 years) | Adulthood (30–76 years) Smoking status (I/Q) | Adulthood (30–76 years) ↑ Prevalence in COPD and noncancerous lung tissue from patients with LADC | Adulthood (30–76 years) Lung tissue Specific genome-wide DNA methylation changes | NA | 152b |
### Table 3 (Continued)

| Environmental exposure | Human cohort                                                                 | Exposure                                                                 | Adverse phenotype                                                                 | Epigenetic change                                                                 | Correlation with gene expression | Reference |
|------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|---------------------------------|-----------|
| Smoking                | Third Xiangya Hospital of Central South University patients \(n = 130, \text{mean age 57 years}\) | Adulthood (40–75 years) Smoking status (I/Q)                             | Adulthood (40–75 years) ↑ Risk of COPD development                               | Adulthood (40–75 years) Serum ↑ miR-21 and ↓ miR-181a                          | NA                               | 153bc     |
| Smoking                | ESTHER cohort \(n = 3,588, \text{mean age 62 years}\)                       | Adulthood (50–75 years) Smoking status (I/Q)                             | Adulthood (50–75 years) ↑ Mortality (including cardiovascular and cancer mortality) | Adulthood (50–75 years) Blood ↓ Intragenic F2RL3 DNA methylation               | NA                               | 154       |
| Smoking                | MESA cohort \(n = 1,264, \text{mean age 70 years}\)                         | Adulthood (55–94 years) Smoking status (I/Q and urinary cotinine)        | Adulthood (55–94 years) CVD (↑ carotid plaque score)                             | Adulthood (55–94 years) Blood (monocytes) ↓ Intragenic AHR DNA methylation     | Yes                              | 155       |
| Smoking                | Fred Hutchinson Cancer Research Center patients \(n = 523, \text{mean age 58 years}\) | Adulthood (35–74 years) Smoking status (I/Q)                             | Adulthood (35–74 years) ↑ Risk of recurrence and lethal disease                 | Adulthood (35–74 years) Tumor tissue Specific genome-wide-DNA methylation changes | Yes                              | 156       |
| SHS                    | TRR cohort \(n = 21\) monozygotic twin pairs discordant for asthma, mean age 33 years | Childhood-adulthood (9–76 years) SHS exposure (I/Q)                      | Childhood-adulthood (9–76 years) Asthma (↓ T cell function)                     | Childhood-adulthood (9–76 years) Blood (Treg/Teffs) ↑ FOXP3 promoter and intronic DNA methylation in Tregs ↑ IFNγ promoter DNA methylation in Teffs | Yes (protein level)              | 157b      |

(Continued)
Table 3 (Continued)

| Environmental exposure | Human cohort | Measurements (stage of development and type) | Correlation with gene expression | Reference |
|-------------------------|-------------|---------------------------------------------|---------------------------------|-----------|
| Wood smoke              | Lovelace smokers’ cohort (n = 1,861, age 40–75 years) | Adulthood Wood smoke exposure (I/Q) | Adulthood ↑ Risk of respiratory disease | Adulthood Sputum (n = 1,267) ↑ p16 and GATA4 promoter DNA methylation | NA | 158 |

*Only studies that measured both adverse phenotypes and associated epigenetic changes in response to an environmental exposure were reviewed. All the studies shown here demonstrated associations between environmental exposure(s), specific epigenetic change(s), and adverse phenotype(s) that were further investigated in a relevant in vivo and/or in vitro system.

*Studies directly sampled the target tissue or validated the same change in the blood and target tissue of an appropriate in vivo model.

‘Studies further investigated associations in a relevant in vivo and/or in vitro system.

Abbreviations: BAL, bronchoalveolar lavage; BAM BAM, Behavioral and Mood in Babies and Mothers; CCCEH, Columbia Center for Children’s Environmental Health; CEAS, Childhood Environment and Allergic disease Study; COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; CVD, cardiovascular disease; DAC, 5-aza-2’-deoxycytidine (DNMT inhibitor); DMR, differentially methylated region; DNMT, DNA methyltransferase; EDC, endocrine-disrupting chemical; ELEMENT, Early Life Exposure in Mexico to Environmental Toxins; EPIC-Turin, European Prospective Investigation into Cancer and Nutrition; ER, estrogen receptor; ESTHER, Epidemiologische Studie zu chancen der verh¨utung, Fr¨uherkennung und optimierten THerapie chronischer ERkrankungen in der ¨alteren Bev ¨olkerung; ETS, environmental tobacco smoke; GECKO, Groningen Expert Center for Kids with Obesity; GW, gestational week; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; HPFS, Health Professionals Follow-up Study; HSCR, Hirschsprung’s disease; INMA, Infancia y Medio Ambiente; I/Q, interview/questionnaire; LADC, lung adenocarcinoma; LIBCS, Long Island Breast Cancer Study Project; LINA, Lifestyle and environmental factors and their Influence on Newborn Allergy risk; NA, not assessed; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses’ Health Study; NJMU, Nanjing Medical University; NSCLC, nonsmall-cell lung cancer; NYU, New York University; PAH, polycyclic aromatic hydrocarbon; PBL, peripheral blood lymphocyte; SHS, secondhand smoke; TBPS, Taiwan birth panel study; Teffs, effector T cells; Tregs, regulator T cells; TRR, Twin Research Registry; TSG, tumor suppressor gene; WBCs, white blood cells.
attention. Growing evidence of the harmful effects of EDCs in wildlife and human populations called for research within experimental systems to explain their observed, long-lasting, nongenotoxic effects. Consequently, rodent and cell models were developed to study these effects. Such model systems included the agouti viable yellow mouse, in which changes in DNA methylation state are reflected in an altered coat color (50). DES was the first EDC demonstrated to induce epigenetically mediated phenotypic changes in an experimental system, followed by a number of other chemicals (reviewed in 50, 51). In the agouti mouse model, both DES and BPA induce DNA methylation changes at the agouti locus, which corresponds with altered expression of the agouti gene and, ultimately, variations in coat color (50). However, a more recent study using the same mouse model could not replicate the finding with BPA (52). Over the past 5 years, the number of studies investigating, and therefore our understanding of, the mechanistic links between EDC exposure and adverse outcomes in experimental systems has increased dramatically. Thus, we limited our PubMed search on the evidence for EDC-induced epigenetic toxicity in such experimental systems to more recent studies (from 2009 onward). This search identified the 51 studies shown in Table 4.

These studies are predominantly focused on rodent models exposed to BPA and altered DNA methylation. However, DNA methylation changes (and some histone and miRNA modifications) following exposure to phthalates, parabens, flame retardants, and dioxins in rodent and other experimental systems, such as primary human cells or advanced cell culture models, have also been demonstrated. A particular set of genes frequently studied in the context of DNA methylation are imprinted genes. EDC-induced DNA methylation changes at imprinted regions are linked mainly to abnormalities in sperm or egg maturation and function, and they appear to affect paternal imprinting more frequently than maternal imprinting. Very few studies address such sex differences. This is surprising considering that EDCs can affect sexual hormone signaling, and differences in the responses of males and females to EDCs have long been observed in wildlife and human populations. Also similar to human epidemiological investigations, studies in experimental systems vary enormously regarding model type, dose, route of exposure, timing of exposure, and correlation of changes with gene expression at the mRNA and/or protein level.

Demonstrating a correlation between specific epigenetic changes and downstream gene expression is vital for building mechanistic evidence that the observed EDC-induced toxicities are epigenetically mediated. Of the 90 studies in wildlife populations, human cohorts, or experimental systems, 53 (59%) demonstrated a correlation between DNA methylation, histone modification, or miRNA expression and downstream gene expression. Correlations in the remaining 37 (41%) were not measured or, in some cases, shown not to exist. Such epigenetic changes may still prove useful as markers of exposure. Likewise, the numerous studies investigating EDC-induced changes without simultaneously assessing any phenotypic endpoints may still provide useful exposure markers and/or mechanistic data. In addition, multiple studies in wildlife populations, human cohorts, and experimental systems report no association between EDC-induced adverse outcomes and epigenetic changes. Such studies are also valuable because they exclude a link between a specific epigenetic change, exposure, and/or the adverse end points/markers investigated.

Nevertheless, numerous studies in both experimental systems and human cohorts support the hypothesis that EDCs can induce specific epigenetic changes that result in later life health effects by providing evidence that (a) DNA methylation changes, and some histone modifications, induced by EDCs in early development (pre, peri-, or neonatally) can persist into later life, and (b) while certain health outcomes can be detected soon after birth, many manifest later or in conjunction with an additional stressor. For example, neonatal exposure of rats to BPA resulted in changes to circulating sex hormones, prostate morphology, and the DNA methylation status of specific genes in prostate tissue (53–56). Upon treatment with testosterone and estradiol, which induce prostate
Table 4  Current evidence for EDC-induced epigenetic changes in experimental systems

| Exposure                          | Model system | Timing | Assessment of effect | Adverse phenotype(s) | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|----------------------------------|--------------|--------|----------------------|----------------------|---------------------------------------------|----------------------------------|--------------|
| **Reproductive health in males** |              |        |                      |                      |                                             |                                  |              |
| Benzo[a]pyrene, 5,10, or 25 mg/kg/day | Rat/male     | PND 1–7| PND 8, 35, 90         | Transient decrease of testis weight Persistent decrease of serum testosterone levels at higher doses | Persistent decrease in H3K14ac at the StAR promoter | Yes                  | 159          |
| BPA: 400 μg/kg/day | Rat/male     | PDN 1–5| F0: PDN 125 F1: GD 20 | Decreased sperm quality and subfertility Postimplantation loss | Hypermethylation of ERα and ERβ in testes Increased Dnmt3a and Dnmt3b protein levels Hypomethylation of Igf2 in sperm Hypomethylation of Igf2 in resorbed embryos (BPA versus control) Hypomethylation of Igf2 in resorbed compared with viable embryos | Yes                  | 160, 161     |
| BPA: 20 μg/kg/day | Mouse/male   | GD 1–5 | PND 50               | Decreased sperm quality Decreased serum and testes testosterone | Decreased H3K14ac at the StAR promoter | Yes                  | 162          |

*(Continued)*
### Table 4  
(Continued)

| Exposure          | Species/sex | Period of exposure | Assessment of effect | Adverse phenotype(s)                                                                 | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|-------------------|-------------|--------------------|----------------------|-------------------------------------------------------------------------------------|---------------------------------------------|-----------------------------------|--------------|
| n-Butylparaben: 64, 160, 400, or 1,000 mg/kg/day | Rat/male    | GD 7 to PND 21     | PND 90               | Increased testis pathology Reduced testosterone and increased estrogen levels Changes in enzymes regulating sex hormone levels in testes | Hypomethylation of ERα in the two highest doses | Yes                  | 163          |
| n-Butylparaben: 64, 160, 400, or 1,000 mg/kg/day | Rat/male    | GD 7 to PND 21     | PND 90               | Increased testis pathology Reduced testosterone and increased estrogen levels Changes in enzymes regulating sex hormone levels in testes | Hypomethylation of ERα in the two highest doses | Yes                  | 163          |
| DEHP: 300 mg/kg/day | Mouse (two different strains: FVB/N and C57BL/6J)/male | GD 9–19            | 8 weeks              | Decreased sperm cells in C57BL/6J but not in FVB/N mice | Strain-specific differences in genome-wide methylation changes upon DEHP treatment for Fkbp1a, Smim8, Praw2, and Tmem123 | Yes                  | 164          |
| Exposure                          | Model system | Timing | Adverse phenotype(s)                                                                 | Correlation with gene expression | Reference(s) |
|----------------------------------|--------------|--------|--------------------------------------------------------------------------------------|----------------------------------|---------------|
| DEHP: 1, 10, or 100 mg/kg/day    | Rat/male     | GD 9–21 | Decreased testes weight Decrease in seminal vesicles Decreased AGD Decreased testosterone and estradiol levels | Increased methylation of Sp1 and SF-1 in Leydig cells Increased expression of Dmnt1, Dmnt3a, and Dmnt3b | Yes           | 164 |
| Nonylphenol: 10 µg/mL            | Murine TM4 Sertoli cell line | 3 or 24 h | 3 or 24 h | Increased cell death and changes in cell function | 9 miRNAs upregulated (miR-125a-3p, miR-297c, miR-421, miR-452, miR-483, miR-574-3p, miR-574-5p, miR-669a, miR-720) and 11 downregulated (let-7g, miR-107, miR-10a, miR-15a, miR-15b, miR-199b*, miR-26a, miR-29c, miR-324-5p, miR-331-3p, miR-342-3p) at both time points | NA            | 165 |

(Continued)
| Exposure                      | Species/sex                      | Period of exposure | Timing               | Adverse phenotype(s)                                                                 | Epigenetic change(s)                                                                 | Correlation with gene expression | Reference(s) |
|-------------------------------|----------------------------------|--------------------|----------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|----------------------------------|---------------|
| BPA: 3 and 300 nM             | Murine follicle culture          | 1–12/13 days       | Day 12/13            | Increased survival in BPA3 group                                                      | Hypomethylation of maternally imprinted genes (Igf2r, Sorp, Mst) in BPA3 group, no effects on paternally imprinted genes Reduced H3K9m3 staining in MII oocytes in BPA3 group | NA                               | 166           |
| BPA: 20 or 40 μg/kg/day       | Mice/female Oocyte culture       | PND 7–14 or PND 5–20 | PND 15 or 21         | Primary follicle depletion Inhibition of oocyte maturation Abnormalities in spindle formation during meiosis | Hypomethylation of the paternally expressed imprinted genes Igf2r and Peg3 No changes in maternally expressed H19 Decreased Dnmt expression | NA                               | 167           |
| BPA: 0.5 mg/kg/day            | Pig/female                       | GD 30–90           | GD 65 and 90         | Effect on cyclicity in adult ewes                                                     | 45 miRNAs downregulated at GD 65 11 miRNAs downregulated at GD 90 miR–203 downregulated at both times Potential targets are Srr genes and genes involved in insulin signaling | NA                               | 168, 169     |

(Continued)
### Table 4 (Continued)

| Exposure | Species/sex | Period of exposure | Assessment of effect | Adverse phenotype(s) | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|----------|-------------|--------------------|----------------------|----------------------|---------------------------------------------|----------------------------------|--------------|
| BPA: 25 ng/μL | Placental cell lines 3A (first trimester villous cells), TCL-1 (first trimester extravillous cells), and HTR-8 (third trimester extravillous cells) | 6 days | Day 6 | Cells overexpressing BPA-induced miR-146a are less proliferative and more sensitive to bleomycin (DNA-damaging agent) | miRNA microarray: 21 miRNAs differentially expressed in both HTR-8 (total 60) and 3A (total 25). No changes in TCL-1. Validation for let-7f, let-7g, miR-21, and miR-146a | NA | 170 |
| BPA: 0.6, 52 μg/kg/day | Rat/female | GD 9 to PND 21 | Pregnancy and lactation (F1) | Decline in milk production and changed milk composition | DNA methylation and histone modifications expression of histone modifiers | Yes | 171 |
| DEHP: 20 or 40 μg/kg/day | Mouse/female Oocyte culture | PDN 7-14 or PDN 5-20 | PDN 15 or 21 | Depletion of primordial follicles. Defects in spindle formation | Hypomethylation of Igf2r and Peg3. Decreased Dnmt3b and Dnmt3l | NA | 172 |
| MEHP: 50, 100, 180 μM | HTR-8/SVneo placental cell line (first trimester) | 4, 8, 24, and 48 h | 4, 8, 24, and 48 h | Decreased cell viability and increased apoptosis after 72-h treatment with higher doses. Increased ROS production for all doses at all time points | Increased preeclampsia-associated miR-16 | Decreased miR-16 target Bcl-2, which was not observed when miR-16 was knocked down | 173 |
| Exposure                                      | Species/sex | Period of exposure | Timing | Assessment of effect | Adverse phenotype(s) | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|----------------------------------------------|-------------|--------------------|--------|----------------------|----------------------|--------------------------------------------|---------------------------------|--------------|
| 4-Hydroxy-2,4,6-trichlorobiphenyl (PCB-F): 100 and 200 μg | Slider turtles | Stage 16           |        | Stages 16, 17, and 19 | 75–90% female hatching | Decreased number of oviducts | Changes in cyp19a1 methylation at specific CpGs, but no clear pattern | Increased cyp19a1 expression | 174          |
| PCB Aroclor 1254: 2.5, 12.5 and 62.5 μM     | In vitro implantation model using human endometrial cells (Ishikawa) and JAR cell spheroids | 48 h    | 48 h              | Decreased spheroid attachment at higher doses Increased outgrowth of the spheroids at highest dose Activation of epithelial-mesenchymal transition Phenotypes were rescued upon miR-30d overexpression | Decreased miR-30d expression | miR-30d target Snai1 upregulation |                             | 175          |

**Growth and metabolism**

| Exposure                  | Species/sex                  | Period of exposure | Timing | Assessment of effect | Adverse phenotype(s) | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|---------------------------|------------------------------|--------------------|--------|----------------------|----------------------|--------------------------------------------|---------------------------------|--------------|
| BDE-47: 25 μM             | Murine preadipocytes (3T3-L1) differentiation | Days 1–8          | Day 8  | Increased adipogenesis | Hypomethylation of Pparg2 | Yes                          |                                | 176          |

(Continued)
| Exposure          | Species/sex                     | Period of exposure | Assessment of effect | Adverse phenotype(s)                                                                                           | Correlation with gene expression | Reference(s) |
|-------------------|--------------------------------|--------------------|----------------------|---------------------------------------------------------------------------------------------------------------|----------------------------------|---------------|
| BPA: 2.5 μg/L     | Mouse/male                     | PND 1 until sacrifice | 8 weeks or 10 months | Increased body weight and amount of fat tissue at 10 months Hepatic lipid accumulation                         | Reduced DNA methylation of genes involved in fatty acid and cholesterol metabolism (*Srebf1*, *Srebf2*, *Fasn*, *Hmgrc*) in liver Reduced *Dnmt1*, *Dnmt3a*, and *Dnmt3b* in liver levels | Yes; 177       |
| DEHP: 1, 10, 100 mg/kg/day | Rat/male and female | GD 9–21             | PND 60               | Increased blood glucose Decreased insulin sensitivity Increased fat weight Decreased insulin binding and changes in insulin signaling Impaired glucose uptake in gastrocnemius muscle | Hypermethylation of *glut4* in gastrocnemius muscle Increased *Dnmt1*, *Dnmt3a*, and *Dnmt3b* expression | Yes; 178 |
| Cancer            | BPA: 2, 10, or 50 μg/kg/day    | Rat/male            | PND 1–5              | Increased precursor lesion of prostate cancer upon adult testosterone + estradiol treatment                      | Hypomethylation of *Sgk2a* Increased H3 K4m3 at Grb2, Igrp1, Nfk-bas, Map2k2, Mapk3, Pdpk, Ikbkb, Erk62, Pdgfb, Hras, Cac1, Hsp90ab1 | Yes; 53, 68 |

*(Continued)*
### Table 4  (Continued)

| Exposure          | Species/sex       | Period of exposure | Assessment of effect | Epigenetic change(s) (F, females; M, males)                                                                 | Correlation with gene expression | Reference(s) |
|-------------------|-------------------|--------------------|----------------------|-------------------------------------------------------------------------------------------------------------|----------------------------------|--------------|
| BPA: 10 μg/kg/day | Rat/male          | PND 1–5           | PND 70, 90, and 120  | Increased precursor lesion of prostate cancer upon adult testosterone + estradiol treatment                   | Yes                              | 54–56, 68    |
|                   |                   |                    |                      | Genome-wide DNA methylation changes                                                                        |                                  |              |
|                   |                   |                    |                      | Hypermethylation of Tpd52, Creb3l4, Paoqr4, Hpcal1                                                        |                                  |              |
|                   |                   |                    |                      | Hypomethylation of Sox2, Pde44, Nisp1                                                                      |                                  |              |
|                   |                   |                    |                      | Increased expression of Dnmt3a, Dnmt3b, Mbd2                                                              |                                  |              |
|                   |                   |                    |                      | Increased H3K4me3 at Grb2, Igr1r, Nfk-ba, Map2k2, Mapk3, Pak2k, Ikkb, Erk2, Pdgp, Hras, Cencl, Hip90ab1 |                                  |              |
| BPA: 1 or 10 μM   | Normal-like human breast epithelial cell line MCF-10F | 2 weeks             | 2 weeks              | Increased tubule formation                                                                                    | Yes                              | 179          |
|                   |                   |                    |                      | Increased sphere size                                                                                        |                                  |              |
|                   |                   |                    |                      | Genome-wide changes in DNA methylation and global gene expression changes                                   |                                  |              |
|                   |                   |                    |                      | Hypermethylated and downregulated for both doses: PARD6G, FOXP1, SFRS11, ELL2, BTN3A2                    |                                  |              |
|                   |                   |                    |                      | Hypomethylated and upregulated for both doses: MLL, NUP98, BOLA3                                            |                                  |              |

(Continued)
| Model system | Timing | Period of exposure | Species/sex | Exposure | Correlation with gene expression | Epigenetic change(s) | Adverse phenotype(s) | Reference(s) |
|--------------|--------|-------------------|-------------|----------|---------------------------------|---------------------|----------------------|--------------|
|              |        |                   | Human mammary epithelial cells | BPA: 10 or 100 nM | NA | Hypermethylation of the cancer-related genes BRCA1, CCNA2, CDKN2A, TNFRSF10B, TNFRSF10D, HIC1 | External proliferation rate increased, increased sphere size increased, increased senescence | Hypermethylation of BRCA1, CCNA2, CDKN2A, THBS1, TNFRSF10B, TNFRSF10D, HIC1 | 180 |
|              |        |                   | Mouse/female | BPA: 50 ng, 50 μg, or 50 mg/kg/day | NA | Genome-wide DNA methylation changes | Dose-dependent increased incidence of hepatic tumors | Hypermethylation of Myh7b, Hypomethylation of Slc22a12 | 181, 182 |
|              |        |                   | Mouse/female | DES: 1 mg/kg/day | NA | Persistent decrease of Tet1 expression and global 5-hydroxymethylcytosine | Uterine cancer after second hit by E2 treatment | Hypermethylation of Six1 | 63 |
|              |        |                   | Murine Y1 adrenocortical tumor cell (Y1) and the mouse Leydig tumor cells (MLTC-1) | MRP: 1 μM | NA | Increased progesterone production | Increased Dna1 expression, Decreased Dnmt3a and Dnmt3b expression | Increased vimentin production, increased vimentin expression | 183 |
| Exposure | Species/sex                                      | Model system | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|----------|------------------------------------------------|--------------|---------------------------------------------|---------------------------------|--------------|
| MBP: 0.1, 1, 10, or 100 μM | Murine Y1 adrenocortical tumor cells (Y1) and the mouse Leydig tumor cells (MLTC-1) | Period of exposure | 24 h | Assessment of effect | 24 h | Adverse phenotype(s) | Decreased miR-200c levels | NA | 184 |
| 3,3′,4,4′,5′-Pentachloro-phenyl (CB126): 0.3, 3, or 30 μM | Human endometrial stromal cells | 48 h | 48 h | Increased E2 production | Increased expression of inflammatory markers | Reduced (dose-dependent) DNA methylation at the 17-beta-hydroxysteroid dehydrogenase gene | Yes | 185 |
| TCDD: 1 μg/kg | Rat/female | GD 15 alone or in combination with resveratrol at GD 7 | PND 46, 71 | Increased proliferative structures in mammary tissue at PND 71, counteracted by resveratrol | Hypermethylation of BRCA-1 in mammary tissue at both time points, counteracted by resveratrol | Yes; recruitment of AhR and Dnmt1 also increased, counteracted by resveratrol | 77 |
| TCDD: 50 μg/kg | Mouse/male | 9 weeks | 2 and 14 days after injection | Liver damage (apparent 6 days after injection) that was mitigated by administration of the Cox2 inhibitor NS-389 | Decreased levels of miR-101a | Increased levels of miR-101a target Cox2 | 186 |
| Exposure | Species/sex | Period of exposure | Assessment of effect | Epigenetic change(s) | Correlation with gene expression | Reference(s) |
|----------|-------------|--------------------|---------------------|---------------------|----------------------------------|---------------|
| BPA: 40 μg/kg/day | Rat/male and female | GD 1 to PND 21 | PND 46 | Sexual dimorphic changes in HPA axis and anxiety-like behavior | Hypermethylation of Fkbp5 | Yes | 75 |
| BPA: 200 μg/kg/day | Mouse/male and female | GD 0–19 | PND 28 and 60 | Decreased novel object exploration in males resulting in significant difference between males and females in the treatment group | In males, hypermethylation of Bdnf and Grin2b in hippocampus and blood Hypermethylation of homologous Bdnf region in human cord blood from mothers with high pregnancy BPA levels | Yes, for the mouse findings | 124 |
| BPA: 20 μg/kg/day | Mouse/male and female | GD 0–19 | PND 28 | Sexual dimorphic changes in social, exploratory, and anxiety-like behavior | In males, hypermethylation of Esr1 prefrontal cortex In females, hypomethylation of Esr1 in hypothalamus | No clear result | 187 |
| PCB 95: 200 nM | Rat primary hippocampal cells | 2 h | 2 h | Increased synaptogenesis, mitigated by knockdown of miR-132 | Increased levels of miR-132 | Decreased levels of miR-132 target p250GAP | 188 |

(Continued)
| Exposure | Species/sex | Period of exposure | Assessment of effect | Adverse phenotype(s) | Epigenetic change(s) (F, females; M, males) | Correlation with gene expression | Reference(s) |
|----------|-------------|--------------------|---------------------|----------------------|---------------------------------------------|---------------------------------|--------------|
| **Heart function** | | | | | | | |
| BPA: 50 μg/kg/day | Rat/male | PND 21 to week 24 or 48 | Week 24 or 48 | Myocardium hypertrophy | Hypermethylation of PGC-1α and increased Dnmt1 and Dnmt3b expression | Yes | 189 |
| BPA: 50 μg/kg/day | Rat/males and females | GD 11.5 to 4 months GD 11.5 to PND 21 | 4 months | BPA5: increased growth Sex-specific differences in heart function and blood pressure BPA5: increased prostate weight Smaller females Sex-specific differences in heart function and blood pressure Increased AGD | In females, hypermethylation of Casq2 In males, increased Dnmt3a and decreased Dnmt3b protein levels In females, decreased Dnmt3a levels In females, hypermethylation of Casq2 In males, increased Dnmt3a and decreased Dnmt3b protein levels In females, decreased Dnmt3a levels | Yes (protein levels) | 190 |
| OR: 200 μg/kg/day | | | | | | | |
| Exposure                        | Species/sex | Period of exposure | Assessment of effect | Adverse phenotype(s)                                                                 | Epigenetic change(s)                                                                 | Correlation with gene expression | Reference(s) |
|--------------------------------|-------------|--------------------|----------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------|--------------|
| DES: 0.1, 1, or 10 μg/kg/day   | Mouse/male  | GD 11.5–14.5       | 4 months             | Systolic dysfunction and increased diastolic relaxation after swim training in higher DES doses | Hypermethylation of Casq2 in 1 μg/kg/day dose in heart Increased Dnmt3a protein levels | Yes, direct (protein levels)      | 191, 192     |
| Immune function                |             |                    |                      |                                                                                      |                                                                                      |                                  |              |
| Phenanthrene compared with TCDD: 300 nM | Human Treg  | 1–7 days           | 1–7 days             | Decreased Treg function Increased differentiation to effector T cells (Th2) compared with TCDD that induces differentiation to (Th17) | Hypermethylation of FOXP3, which becomes irreversible upon 3 days treatment TCDD does not induce hypermethylation Increased DNMT1 and DNMT3a | Yes                  | 61           |
| TCDD: 10 μg/kg                 | Mouse/male  | GD 14              | GD 17                | Increased apoptosis of thymocytes                                                   | Upregulation of miR-122 and miR-181a Downregulation of miR-23a, miR-18b, miR-31, miR-182, and mm7-let-7e | Upregulation of Cyp1a1 (possible target of miR-31), Ab/R (possible target of miR-182), Fas (possible target of miR-23a), FasL (mm7-let-7e target) | 193, 194     |

(Continued)
### Table 4 (Continued)

| Exposure         | Species/sex          | Period of exposure | Assessment of effect | Adverse phenotype(s)                                      | Epigenetic change(s) (F, females; M, males)                                      | Correlation with gene expression                                      | Reference(s) |
|------------------|----------------------|--------------------|----------------------|-----------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------|---------------|
| **Embryonic development** |
| BPA: 0.04, 1, 25, or 100 μM | Mouse embryoid bodies | 2, 4, and 6 days | 2, 4, and 6 days | Changes in differentiation capacity: increased endodermal and mesodermal, decreased ectodermal differentiation | Decreased miR-134 at higher doses at all time points | Increased miR-134 targets Oct4, Nanog, and Sox2 | 195 |
| Triclosan: 0.01, 0.1, 1, 10, or 50 μM | Mouse embryonic stem cells | 24 h | 24 h | Increased apoptosis Reduced pluripotency | Dose-dependent increase in miR-134 | Decreased miR-134 targets Oct4, Nanog, Sox2 | 196 |
| TCDD: 64 μL/kg/day | Mouse/male and female | GD 12–17 | GD12, 13, 13.5, 14, 14.5, 15, 15.5, 16 and 17 excluding GD 16.5 | Cleft palate formation | Changes in lncRNA h19 levels in palate tissue: decrease at GD 13.5, increase at GD 14.5 | Inverse changes in IGF2 expression (target of lncRNA h19) | 197 |

*Only studies that measured both adverse phenotypes and associated epigenetic changes in response to EDC exposure were reviewed. All the studies shown here demonstrated correlations among exposure(s), specific epigenetic changes(s), and adverse phenotype(s).*
cancer in rats, the severity of prostatic lesions was greater in the animals neonatally exposed to BPA. These studies also demonstrate the potential of epigenetic changes to act as markers of disease or disease susceptibility, detectable much earlier than the adversity itself. Only a few studies describe persistent histone modifications in the context of EDC exposure. This is most likely because analysis of this epigenetic mark is more challenging than measurement of DNA methylation and ncRNA expression. Furthermore, these marks are studied mainly in the context of direct gene transcription, which does not yield information on their persistence over time.

Finally, recent findings also suggest that the genetic background of the experimental system influences health outcomes and epigenetic changes. Comparing two different mouse strains, the severity of effects on male fertility and changes to spermatozoal DNA methylation patterns following exposure to the plasticizer bis(2-ethylhexyl) phthalate (DEHP) varied significantly between the strains (57). Such differences in underlying genetics are also likely to contribute to the observed variability of human individuals and populations to EDC exposures.

3.3. How EDCs Can Perturb Epigenetic Regulation

While it has become clear that EDCs can induce epigenetic changes, how they induce these alterations remains obscure. This is partly due to the fact that some major questions regarding the regulation of epigenetic processes remain unsolved. For example, it is unclear how epigenetic marks are targeted to specific DNA regions or how different epigenetic mechanisms (i.e., DNA methylation, histone modifications, ncRNAs) interact with each other. Numerous studies report general effects of EDCs on the epigenetic machinery, whereas region-specific changes are less commonly investigated. Emerging mechanisms are summarized in the following sections and in Figure 3.

3.3.1. General epigenetic mechanisms: effects on the epigenetic machinery. Exposure to EDCs induces long-lasting effects on the expression pattern of DNMTs that can be transmitted to subsequent generations (see Table 4 and Section 3.6). For example, exposure to vinclozolin (58) or methoxychlor (59) can induce effects on DNMT expression across multiple generations in rats. In some studies, changes in DNMT expression are concomitant with an overall change in DNA methylation across the genome, which is the most likely consequence of such a general effect. However, in many other studies, DNMT expression changes were connected to transcriptional changes resulting in altered levels of mRNAs and/or microRNAs can then lead to altered expression of various components of the epigenetic machinery, including those responsible for DNA methylation (DNMTs, SAM, and other methyl donors), DNA demethylation (TET proteins and TDG), and histone modification (histone modifiers such as HDACs or HATs as well as SAM or other methyl donors). In addition, EDC-activated nuclear receptors can interact with DNMTs, TDG, or histone modifiers. Some EDCs also directly alter the enzymatic activity and/or concentrations of DNMTs, TETs, or histone modifiers to induce changes in DNA methylation and/or histone modification. Inhibitory and activating processes are shown in red and green, respectively.

Figure 3
Potential mechanisms for EDC-induced epigenetic perturbation. EDCs can induce multiple epigenetic changes, including altered DNA methylation, histone modification, and ncRNA expression. Some EDCs interact with nuclear receptors such as the ERs, AR, aryl hydrocarbon receptor, retinoic acid receptor, and/or glucocorticoid receptor, causing them to be released from chaperone complexes. Released nuclear receptors then form homo- or heterodimers (with other receptors or proteins), which then bind to receptor binding sites within the hormone responsive elements of promoter regions and combine with other coregulators to activate or repress transcription. Transcriptional changes resulting in altered levels of mRNAs and/or microRNAs can then lead to altered expression of various components of the epigenetic machinery, including those responsible for DNA methylation (DNMTs, SAM, and other methyl donors), DNA demethylation (TET proteins and TDG), and histone modification (histone modifiers such as HDACs or HATs as well as SAM or other methyl donors). In addition, EDC-activated nuclear receptors can interact with DNMTs, TDG, or histone modifiers. Some EDCs also directly alter the enzymatic activity and/or concentrations of DNMTs, TETs, or histone modifiers to induce changes in DNA methylation and/or histone modification. Inhibitory and activating processes are shown in red and green, respectively. Abbreviations: AR, androgen receptor; CpG, cytosine-phosphate-guanine dinucleotide; DNMT, DNA methyltransferase; EDC, endocrine disrupting chemical; ER, estrogen receptor; HDAC, histone deacetylase; mRNA, messenger RNA; ncRNA, noncoding RNA; SAM, S-adenosylmethionine; TDG, thymine DNA glycosylase; TET, ten-eleven translocation methylcytosine dioxygenase.
gene-specific methylation changes. How this specificity is controlled remains unclear. Furthermore, only a few studies have interrogated the causal effect altered DNMT expression has on the resulting gene expression and phenotypic changes observed upon EDC exposure. One example is a study performed in rats that showed alterations in anxiety behavior and synaptic plasticity following perinatal exposure to BPA (60). These changes were linked to increased expression of \( Dnmt1 \) mRNA and concomitant decreased expression of \( Gad67 \) mRNA, an enzyme important for
GABAergic inhibition in the amygdala brain region. Interestingly, the authors (60) found that administration of the DNMT inhibitor 5-aza-deoxycytidine directly into the amygdala restored Gad67 mRNA expression as well as behavior in BPA-treated rats. Also rarely addressed is how EDCs affect DNMT expression, although an obvious explanation would be direct transcriptional regulation via receptors targeted by the EDC. Indeed, the T cells of AhR-knockout mice display decreased Dnmt1 and Dnmt3b mRNA expression compared with wild-type mice (61). Alternatively, DNMT expression can be altered through the direct action of miRNAs that target Dnmt mRNAs, such as the miR-29 family (62).

While the enzymes for DNA methylation were identified decades ago, the process of DNA demethylation remained enigmatic until recently. Only in the past five years has it become clear that active DNA demethylation is catalyzed by the cooperation of several enzymes, namely TETs and TDG, together with the base excision repair machinery (30). Consequently, very few studies have investigated the effects of EDCs on these enzymes. In one example, neonatal exposure to DES decreased both Tet1 mRNA expression and 5hmC across the genome in the uterus of adult mice (63). In another study, BPA treatment reduced the nuclear localization of TET2 protein in murine gonadotropin-releasing hormone (GnRH) neurons in vitro, which correlated with decreased H3K4 trimethylation at the GnRH promoter and decreased GnRH protein expression (64). Effects on DNA methylation were not assessed.

Changes to histone-modifying enzymes, particularly HDACs and HMTs, following exposure to EDCs have also been reported. Increased expression of HDACs was shown to mediate dioxin-induced effects on pituitary gonadotropins in rats (65). Molecular binding studies provide evidence that phthalates, including benzyl-butyl phthalate (BBP), can bind to sirtuins (SIRT1 and 3), a sub-family of HDACs. Consequently, BBP decreased the expression of Sirt1 and Sirt3 genes, SIRT1 and SIRT3 proteins, and two SIRT-dependent mitochondrial biogenesis genes in HepG2 cells, leading to increased production of reactive oxygen species (66). In addition, BPA-induced histone demethylation via phosphorylation and inhibition of the repressing histone methyltransferase EZH2 has been reported (67). Conversely, BPA-induced histone methylation via phosphorylation and activation of the histone-activating methyltransferase MLL1 has also been shown (68). Increased phosphorylation was due to NR-independent activation of the PI3K/AKT signaling by BPA (67, 68).

EDCs affect not only the enzymes regulating epigenetic modifications but also the levels of their cofactors, in particular S-adenosylmethionine (SAM). SAM is the methyl donor for virtually all methylation reactions in the cell, including methylation of DNA and histone tails. Processes that deplete intracellular SAM thus lead to a global reduction of methylation capacity. SAM is enzymatically produced from methionine obtained through dietary sources, catalyzed by methionine adenosyltransferase (MAT). Methylation of a substrate results in conversion of SAM to S-adenosylhomocysteine, which is recycled back to SAM via homocysteine and methionine, forming the methionine cycle. Aromatic hydrocarbon 3-methylcholanthrene reduces Mat mRNA and MAT protein after in vivo exposure (69). In addition, dietary methyl donors such as folic acid mitigated DNA hypomethylation induced by BPA in the agouti mouse model (70), suggesting that SAM production is involved in the epigenetic effects of BPA. This is supported by a study conducted in the testes of the rare minnow Gobiocypris rarus showing that BPA affected SAM levels (71).

3.3.2. Specific epigenetic mechanisms: effects on defined DNA regions. As mentioned above, an open question in the epigenetics field is how factors regulating epigenetic patterns are targeted to defined genomic regions. This is particularly true for regulators of DNA methylation. Sequence-specific transcription factors as well as ncRNAs may be involved in this process.
Interestingly, among the transcription factors implicated in the regulation of epigenetic patterns, we find well-known EDC-associated NRs. For example, the AR and ER regulate chromatin states (72). Moreover, additional studies have demonstrated that the glucocorticoid receptor, AR, ERs, retinoic acid receptor, and AhR are involved in regulating gene-specific DNA methylation patterns by interacting with DNMTs (73) or TDG (74). Thus, interference with this function of NRs could, at least partly, underlie the epigenetic effects induced by EDCs. Indeed, the effect of BPA on DNA methylation of the *Fkbp5* gene was found to be ERβ dependent (75). Genome-wide DNA methylation analysis in the reproductive tract of adult female mice perinatally exposed to BPA identified more than 1,700 differentially methylated regions, 93% of which overlapped with ERα binding sites (76). In addition, the AhR co-recruits DNMT1 to the *Brcat-1* promoter, which coincides with increased methylation and decreased expression of the *Brcat-1* gene in mammary tissue of mice prenatally exposed to tetrachlorodibenzo-p-dioxin (TCDD) (77).

Few studies have addressed how EDCs regulate ncRNAs. Again, an obvious mechanism would be direct regulation of ncRNA expression via NRs. EDC effects on both ER-mediated regulation of miRNA expression (reviewed in 78) and the lncRNA HOTAIR (79) provide evidence for such direct mechanisms. Likewise, AhR activation by polycyclic aryl hydrocarbons directly increases expression of miR-25, which is upregulated in different cancers and targets the tumor suppressor gene *p53* (80).

### 3.4. Transgenerational Epigenetic Inheritance of EDC-Induced Changes

Transgenerational epigenetic inheritance (TEI) is a mechanism by which parental environmental exposures could induce phenotypic variations in subsequent progeny. Two fundamental events are required for TEI to occur: 

(a) Environmental exposures must alter epigenetic patterns within the germ line [the series of cells from which the mature gametes (egg or sperm) are derived; see Section 3.1], and 

(b) these alterations must be passed onto subsequent generations through reproduction.

These environmentally induced epigenetic alterations in the germ line will then influence the epigenetic state of somatic cells (all other cells not of germ line origin) in offspring and, ultimately, their phenotype (81).

For a full understanding of TEI, however, it is important to make the distinction between mitotic and meiotic epigenetic inheritance. Mitotic epigenetic inheritance describes maintenance of the somatic mother cell’s epigenetic patterns (e.g., DNA methylation) in the resulting somatic daughter cells. In contrast, meiotic epigenetic inheritance describes maintenance of the parental germ cells’ epigenetic patterns in their progeny’s germ cells. Disruption in mitotic epigenetic inheritance can lead to diseases in the exposed individual, but since this is a somatic process, it will not affect future generations. Disturbances in meiotic epigenetic inheritance, however, can have consequences that transcend generations owing to the involvement of the germ line. Environmentally induced alterations in the germ line, whether genetic or epigenetic, will be transmitted to subsequent generations and can induce altered phenotypes in the progeny. Epigenetic changes in the germ line can be generated by direct actions of environmental exposures (including EDCs) that cross the uterine and/or placental barriers or by indirect actions of exposures that alter the physiological responses of somatic cells in close contact with germ cells (such as the testicular Sertoli cells).

The distinction between epigenetic effects in somatic cells compared to those in germ cells is important when interpreting results that emerge from transgenerational experiments. In the strictest sense, a transgenerational effect is visible only in the first generation in which the germ line was not developmentally exposed to the environmental factor being studied. Because embryonic exposure can generate both somatic and germ line epigenetic effects, first-generation (F1) effects...
Developing germ-line environmental insult

Somatic epigenetic effects ≠ Germ-line epigenetic effects

Figure 4
Schematic representation showing why phenotypic effects can differ in the F1 or F3 generation after developmental exposure to environmental toxicants: phenotypic effects observed in the F1 (e.g., disease phenotypes) derive from somatic epigenetic modifications, whereas phenotypic effects observed in the F3 derive from germ-line epigenetic modifications. These F3 phenotypic effects, in which germ-line epigenetic alterations are involved, are considered to be transmitted through transgenerational epigenetic inheritance. Figure is adapted from 82.

often differ from third-generation (F3) effects. This is due to the fact that the F1 phenotype will express somatic epigenetic changes, whereas the F3 phenotype will exhibit effects derived from germ line epigenetic alterations, which were induced in the ancestor and transgenerationally transmitted (Figure 4). Here we describe the advances to date that show or suggest TEI in this strictest sense, i.e., when epigenetic effects are observed in the germ line after environmental exposures and are transmitted to future generations, influencing phenotype formation and mitotic epigenetic processes in the offspring. There is often some confusion and inappropriate application of the term “transgenerational study” in the scientific literature, such that much of the literature does not represent true TEI.

Initial evidence in animal models indicating that in utero exposure to EDCs could generate transgenerationally transmitted phenotypes was obtained from a study in 2000 on the reproductive effects of DES. Administration of DES to pregnant rats during early post-implantation development generated reproductive abnormalities in subsequent generations (83). These abnormalities included increased susceptibility for proliferative lesions in the rete testis (usually of uncommon occurrence) and increased tumor formation in other reproductive tract tissues in F1 and F2 male offspring (83). The authors suggested that epigenetic alterations could be involved in the generation of these reproductive abnormalities and their transmission to future generations.

In 2005, researchers first described an environmentally induced transgenerational inheritance process that was related to epigenetic changes in the germ line and resulted from in utero exposure
to vinclozolin, a known EDC (84). Vinclozolin, an antiandrogenic fungicide, had previously been shown to increase apoptosis in male germ cells of offspring whose mothers were treated during early pregnancy (85). Further experiments demonstrated that this increased apoptosis was also observed in the next three generations in the absence of any further exposure (84, 86, 87). Epigenetic changes in the germ line were subsequently detected three generations after the initial maternal (ancestral) exposure to vinclozolin (84, 88, 89). An independent study has also reported that vinclozolin induces transgenerational epigenetic changes in imprinted genes within sperm (90).

With regard to other exposures generating germ line epigenetic effects, in utero exposure to TCDD decreased hepatic Igf2 expression and altered DNA methylation in the imprinting control region associated with transcriptional regulation of Igf2 in a transgenerational fashion (both in F1 and F3 animals). Likewise, in utero exposure of rats to dichlorodiphenyldichloroethylene (a degradation product of DDT) resulted in decreased Igf2 and increased H19 expression, together with altered DNA methylation of the H19/Igf2 cluster imprinting control region, in sperm from F1 and F3 (91). In addition, transgenerational changes in the expression of DNMT3A and DNMT3B, but not DNMT1, have been observed in rats following ancestral treatment with TCDD (92). These findings represent independent confirmation that transgenerational epigenetic effects in the germ line can be induced by early-life exposure to EDCs.

Interestingly, DNA methylation and expression patterns are disrupted in PGCs when maternal exposure to vinclozolin occurred between the onset of gonadal sex determination and testis cord formation (93). These alterations were maintained in subsequent generations (58, 93) and could influence the epigenome of the mature sperm. Further experiments determined that, after ancestral exposure to vinclozolin, somatic gene expression and epigenetic patterns were also transgenerationally altered in somatic tissues in close contact with gonadal development, such as Sertoli (94) and granulosa (95) cells.

Since the first description of the transgenerational effects of vinclozolin, other diseases have been shown to be transgenerationally transmitted, and other chemical exposures (including EDCs) have been reported to induce TEI. Transgenerational effects have been observed in both males and females and are exposure dependent. A summary of EDC-induced epigenetic changes and phenotypes that are transgenerationally inherited is shown in Table 5. The fact that all EDCs investigated generated a different set of germ line DNA methylation patterns (96–99) raises the conceptual possibility that the sperm epigenome could be an indicator of both ancestral exposures and susceptibility to disease (97, 99, 100).

In addition to the cases in which transgenerational epigenetic effects have been identified, some studies show EDC-induced transgenerational effects without assessment of epigenetic modifications. For example, in male mice, early developmental exposure to DEHP induced transgenerationally transmitted disruption of testicular germ cell associations, reduced sperm count, and decreased sperm motility (101). In female mice, in utero exposure to TCDD reduced pregnancy outcome in a transgenerational manner (F1–F3) to ~50% of the normal rate (102). Interestingly, transgenerational incidence of pregnancy problems (F1–F3) was also observed in rats after in utero exposure to vinclozolin, increasing from being virtually nonexistent to an incidence of ~10% (103). In addition, female descendants of those exposed in utero to ethinyl estradiol (EE), a synthetic estrogen used in contraceptive pills, showed increased susceptibility to mammary tumors (104). This was accompanied by transgenerational increases in the occurrence of terminal end buds (highly proliferative and invasive structures involved in normal breast development from which chemically induced breast tumors can develop) and DNMT1 activity in mammary glands. Similarly, other studies showed EDC-induced transgenerational epigenetic modifications without assessment of phenotypic outcomes. For example, ancestral vinclozolin exposure in rats resulted

EE: ethinyl estradiol
Table 5  Current evidence for adverse EDC-induced transgenerational epigenetic effects

| Exposure tested | Species | Period of exposure | Generation and cell type analyzed | Abnormalities observed | Epigenetic change(s) | Correlation with gene expression | Reference(s) |
|-----------------|---------|--------------------|-----------------------------------|------------------------|----------------------|----------------------------------|--------------|
| BPA + phthalates (high dose: BPA 50 mg/kg BW/day, DEHP 750 mg/kg BW/day, and DBP 66 mg/kg BW/day; low dose = half of high dose) | Rat     | GD 8–14            | F3, sperm                       | M&F: increased adiposity, F: PCOS, primordial follicle loss, pubertal abnormalities | 197 regions | NA | 97, 198 |
| DDE (100 mg/kg BW/day) | Rat     | GD 8–14            | F1–3, sperm                     | M: testis abnormalities, spermatogenic cell apoptosis | Imprinted DMR at the Igf2/H19 cluster | Yes | 91 |
| DDT (high dose: 25 or 50 mg/kg BW/day) | Rat     | GD 8–14            | F3, sperm                       | M&F: kidney abnormalities, increased adiposity, F: PCOS, M: testis abnormalities | 39 regions | NA | 99 |
| Jet fuel 8 (500 mg/kg BW/day) | Rat     | GD 8–14            | F3, sperm                       | F: PCOS, primordial follicle loss | 33 regions | NA | 97, 199 |
| Methoxychlor (200 mg/kg BW/day) | Rat     | GD 8–14            | F3, sperm                       | M&F: kidney abnormalities, increased adiposity, F: PCOS | 37 regions | NA | 98 |
| Permethrin + DEET (permethrin: 150 mg/kg BW/day; DEET: 40 mg/kg BW/day) | Rat     | GD 8–14            | F3, sperm                       | M: testis abnormalities | 363 regions | NA | 97, 200 |
| TCDD (100 mg/kg BW/day) | Rat     | GD 8–14            | F3, sperm                       | M: kidney and pubertal abnormalities | 50 regions | NA | 97, 201 |

(Continued)
Table 5  (Continued)

| Exposure tested                        | Species | Period of exposure | Generation and cell type analyzed | Abnormalities observed                                                                 | Epigenetic change(s)                                                                 | Correlation with gene expression | Reference(s) |
|----------------------------------------|---------|--------------------|-----------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------|--------------|
| TCDD (200 or 800 ng/kg BW/day)         | Rat     | GD 8–14            | F1 and F3, liver                   | M: liver abnormalities                                                                  | Imprinted gene Igf2 and altered DNMTs expression                                    | Yes                              | 92           |
| Vinclozolin (100 or 200 mg/kg BW/day)  | Rat     | GD 8–15            | F2 and F3, sperm                   | M: spermatogenic cell apoptosis, reduced sperm numbers and motility                     | Within the LPLase gene and around the start site of the cytokine-inducible SH2 protein | NA                               | 84           |
| Vinclozolin (100 mg/kg BW/day)         | Mouse   | GD 7–13            | F3, sperm                          | F: PCOS                                                                                | 68 regions in 66 genes in initial screening; 40 regions confirmed                  | NA                               | 88           |
| Vinclozolin (100 mg/kg BW/day)         | Rat     | GD 8–14            | F3, granulosa cells                | F: PCOS, primordial follicle loss                                                      | 43 regions                                                                          | Yes                              | 95           |
| Vinclozolin (100 mg/kg BW/day)         | Rat     | GD 8–14            | F3, Sertoli cells                  | M: spermatogenic cell apoptosis                                                       | 101 regions                                                                          | Yes                              | 94           |
| Vinclozolin (50 mg/kg BW/day)          | Mouse   | GD 10–18           | F1–3; sperm, tail, liver, skeletal muscle | M: reduced sperm motility (only F1)                                                  | Imprinted genes H19 and Peg3 showed a transgenerational effect in sperm; Peg3 showed a transgenerational effect in tail and liver | NA                               | 90           |

Abbreviations: BPA, bisphenol A; BW, body weight; DBP, dibutyl phthalate; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DEHP, bis(2-ethylhexyl) phthalate; DMR, differentially methylated region; DNMT, DNA methyltransferase; EDC, endocrine disrupting chemical; F, females; GD, gestational day; M, males; NA, not assessed; PCOS, polycystic ovary syndrome; TCDD, tetrachlorodibenzo-p-dioxin.
in DNA methylation changes in the PGCs and sperm of F3 males (89, 93). As mentioned in Section 3.2.3, such studies may provide useful markers of TEI and/or mechanistic data.

Transgenerational effects are not limited to reproductive or metabolic diseases. In utero exposure to EDCs have also been reported to generate behavioral transgenerational effects. F3 female rats from either a control or an ancestrally exposed vinclozolin lineage strongly preferred F3 male rats from the control lineage versus the vinclozolin lineage (105). More recently, in utero exposure of mice to low doses of BPA generated differing social interactions within F1 and F3, although both generations included differences regarding controls (106). Moreover, when tested for olfactory discrimination, BPA-exposed juvenile mice displayed higher levels of investigation than did controls in both F1 and F3 (107). Sustained prenatal-peripubertal exposure to EE induced a dose-dependent increase in sexual behavior in exposed F1 males that persisted until their F4 male offspring (108). In addition, a recent study in mice demonstrated that in utero exposure to the drug valproate induced autism-like behaviors in F1 to F3 (109). Interestingly, concomitant changes in gene expression were observed in brain frontal cortex. Again, the role of epigenetic processes in these phenotypes remains to be determined.

EDC-induced transgenerational effects have also been reported in nonmammalian species. In fish, where exposure of natural populations to EDCs is of great concern, transgenerational effects have been observed in medaka (110) and zebrafish (111). Embryonic exposure of medaka to BPA or EE reduced fertilization rates in F2 and embryo survival in F3 (110). Juvenile exposure of zebrafish to dioxin induced transgenerational skeletal abnormalities, a skewed sex ratio, and increased incidence of immature ovarian follicle degeneration and resorption (111). As briefly discussed in Section 3.2, whether EDC-induced epigenetic mechanisms are involved in ecotoxicology is currently under further investigation.

In addition to early developmental exposures to environmental toxicants, exposures after birth have also been evaluated for their transgenerational effects. Perinatal exposure of rats to BPA impaired sperm production and fertility (112) and induced transgenerational alterations in the expression of steroid receptors and their coregulators in testis (113). Exposure of six-week-old male mice to benzo[a]pyrene generated impairments in several fertility parameters, including testicular malformations and decreased sperm production, which were observed until F2 (114).

Evidence that adult EDC exposures can also result in TEI presents new theoretical challenges (115). Although the mechanism through which paternal exposures affect offspring remains to be elucidated, epigenetic changes, including changes in DNA methylation, histone modification, chromatin structure, or ncRNAs, take place during sperm formation in adults (116–118). Therefore, like the critical windows of development in early life, epigenetic reprogramming that takes place during sperm production may also represent a key stage of development open to perturbation by EDCs. This will certainly be an exciting field of research in the near future.

4. OUTLOOK: USING EPIGENETIC END POINTS FOR IMPROVED EDC RISK ASSESSMENT

It is a challenge to detect effects that are induced by early-life exposures but manifest as health issues later in life, or even in subsequent generations. However, a large body of literature demonstrates that environmental chemicals (including EDCs) can indeed generate such effects, most of which are neglected by current chemical risk assessment procedures. The ever increasing weight of evidence that these chemically induced effects may be epigenetically mediated suggests that epigenetic end points, i.e. alterations in epigenetic modifications specifically linked to an adverse health effect, have great potential to help fill such information gaps within chemical safety testing.
Perhaps not surprisingly, there is great international concern as to whether potential chemical exposures during critical windows of human development induce epigenetic changes that may lead to later life adverse health outcomes (such as infertility, obesity, and cancer) and/or across multiple generations. Consequently, OECD member countries are actively considering how epigenetic end points associated with adversity may be incorporated into chemical safety testing for improved chemical risk assessment.

Although only few wildlife studies yield definitively causal epigenetic evidence, the numerous experimental and epidemiological studies reviewed here show that epigenetic modifications can be induced during early life and can precede adverse phenotypic outcomes. Furthermore, an additional stressor, or second hit, that acts on the same pathway is sometimes required for disease manifestation. Thus, epigenetic changes could serve both as early markers of subsequent disease development and as predictive markers of increased susceptibility to further exposures to chemicals and other agents. For chemical safety testing, epigenetic end points may provide (a) earlier and more sensitive markers of toxicity; (b) novel markers of exposure and/or predictors of future toxicity; and (c) novel markers of toxicities that we currently cannot test definitively, such as delayed effects of low-dose exposures, hormone and nongenotoxic carcinogen (i.e., a carcinogen that has the potential to induce cancer without interacting directly with either DNA or the cellular apparatus involved in the preservation of the integrity of the genome) related cancers, and transgenerational effects. However, multiple issues require consideration before epigenetic end points can be used reliably for improved EDC risk assessment for both human and environmental health protection.

Key challenges include identifying the relevant epigenetic mechanisms and markers of EDC exposure and toxicity and starting to build these elements into mechanistic adverse outcome frameworks. Doing so is complicated by the fact that much work to date simply demonstrates that EDCs can induce epigenetic and/or phenotypic changes. None of the wildlife or human cohort studies to date further established causality. Although a few experimental system studies demonstrated some mechanistic causality, these largely represent exploratory experiments using high (often single) doses, many orders of magnitude above likely ecological or human exposures. In addition, even epigenetic changes in somatic cells or in the germ line that might not correlate functionally with disease can still provide an indication or association of future susceptibilities to adverse health outcomes, which could be used as part of a weight of evidence approach in the development of appropriate models for regulatory use.

Another key challenge is the enormous variation in the current body of literature in terms of scientific quality; reproducibility; and choice of model system, analysis methods, and end points. This variation demonstrates that epigenetic toxicity can be species, strain, and sex dependent. Very few studies have directly addressed either sex differences (sexual dimorphism) or “normal” variation. Small differences in experimental systems and protocols can themselves result in epigenetic changes, unrelated to EDC exposure. In an evolutionary context, epigenetic processes have enabled rapid responses to changing environments. Thus, fully characterizing normal epigenetic variation within a system is important (yet extremely challenging) to determine the actual downstream consequences of epigenetic changes and the threshold above which epigenetic toxicity occurs. Indeed, not all EDC-induced epigenetic changes are detrimental, and under certain conditions, they might even be reversible. Moreover, underlying genetic and biological differences in epigenetic responses exist between humans and laboratory animals, between strains of laboratory rodents, and between domesticated and wild species. For regulatory purposes, focused research demonstrating definitive and reproducible epigenetic mechanisms linking EDC exposure to specific adverse phenotypic outcomes generated through standardized experimental and bioinformatics protocols are essential.
Key common misconceptions also need to be addressed. For example, the assumption that higher doses will always generate the strongest effects can be dangerous when interpreting epigenetic effects related to endocrine disruption because non-monotonic effects are not unusual in hormonal responses. Therefore, non-monotonic responses must be considered to ensure accurate assessment of real effects. The effect of multiple exposures or exposure to multiple chemicals is another unexplored area. Most of the research evaluating epigenetic effects is in response to environmental chemicals, single exposures, and single chemicals. However, this is unrealistic because organisms are exposed to a continuous cocktail of multiple (new and old) contaminants that may interact in infinite and unsuspected ways. When acting through the same pathways, they might induce additive, synergistic, or counteracting effects. Future research paradigms need to start addressing this area as a priority, particularly the potential for epigenetic toxicity resulting from co-exposure to multiple interacting chemicals at levels below those currently regulated.

5. CONCLUSION

This review begins with an introduction to the recent history of our growing concern over the impact of human-made chemical contaminants on sentinel wildlife populations (particularly birds and fish) and, ultimately, on humans. A comprehensive review of the current literature is then provided to demonstrate that the adverse endocrine effects have since become better understood as our knowledge of the underlying epigenetic machinery has improved. With the relatively recent development of more advanced and lower-cost analytical approaches, the epigenetic evidence base, stemming from human epidemiological and experimental systems, has greatly improved.

While more mechanistic research is needed, it is clear that chemical tests incorporating epigenetic end points with key epigenetic biomarkers have great potential to become the mechanistic tools of choice to predict toxicity and thus better protect both the environment and public health from the adverse effects of EDC exposure. The prospects for in vitro test development and thus the refinement, replacement, and reduction (3Rs) of animals are also substantial. Currently, such epigenetic tests are still in development by the scientific community. However, a closer discourse between researchers and the regulatory community will help generate the tools needed to create a cost-effective, more protective, and preemptive chemical screening process to reduce the risk of human and environmental populations being adversely “marked for life.”

SUMMARY POINTS

1. Observations and research over the past six decades have demonstrated that chemicals interfering with the hormonal system (EDCs) have long-lasting adverse effects on wildlife populations and human health.

2. In the last decade, evidence has accumulated supporting that epigenetic changes (changes in DNA methylation, histone modifications, and ncRNAs) could underlie such long-lasting adverse effects of EDCs.

3. Experimental research in rodents, fish, and other species has demonstrated that EDC-induced adverse effects can be transmitted to unexposed generations, possibly via trans-generational epigenetic inheritance (TEI).

4. While more knowledge is needed about epigenetic mechanisms in general, and EDC-induced epigenetic changes and their link to adversities in particular, epigenetic changes have great potential to become useful for chemical risk assessment as early markers of adversities later on in life or in subsequent generations.
DISCLOSURE STATEMENT

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