Abstract

There is now considerable evidence indicating the potential for endocrine disrupting chemicals to alter the epigenome and for subsets of these epigenomic changes or “epimutations” to be heritably transmitted to offspring in subsequent generations. While there have been many studies indicating how exposure to endocrine disrupting chemicals can disrupt various organs associated with the body’s endocrine systems, there is relatively limited information regarding the relative susceptibility of different specific organs, tissues, or cell types to endocrine disrupting chemical-induced epimutations. Here we review available information about different organs, tissues, cell types, and/or cell lines which have been shown to be susceptible to specific endocrine disrupting chemical-induced epimutations. In addition, we discuss possible mechanisms that may be involved, or impacted by this tissue- or cell type-specific, differential susceptibility to different endocrine disrupting chemicals. Finally, we summarize available information indicating that certain periods of development display elevated susceptibility to endocrine disrupting chemical exposure and we describe how this may affect the extent to which germline epimutations can be transmitted inter- or transgenerationally. We conclude that cell type-specific differential susceptibility to endocrine disrupting chemical-induced epimutagenesis is likely to directly impact the extent to, or manner in, which endocrine disrupting chemical exposure initially induces epigenetic changes to DNA methylation and/or histone modifications, and how these endocrine disrupting chemical-induced epimutations can then subsequently impact gene expression, potentially leading to the development of heritable disease states.

Key words: epimutations; environmental disruptors; epigenetic programing; DNA methylation; histone modifications; tissue-/cell type-specificity; developmental-stage specificity

Introduction

It is now well established that parental lifestyles or experiences, or environmental conditions or exposures have the potential to alter the epigenome. The resulting abnormalities in epigenomic programing have been termed “epimutations” [1] and can be manifested as alterations in genome-wide patterns of DNA methylation [2, 3], histone modifications [4], chromatin structure [5], and/or levels or types of non-coding RNA transcripts [6, 7], all of which can disrupt normal gene expression patterns [8, 9]. A portion of past research regarding environmentally induced epimutagenesis has focused on the wide array of chemical exposures that can disrupt the epigenome. These studies have shown that a specific class of chemicals known as endocrine disrupting chemicals (EDCs) can, in addition to having direct toxic effects on many of the body’s endocrine systems,
alter the epigenome [10]. This has led to studies of specific EDCs to better understand the extent to, and mechanisms by, which these chemicals function to disrupt normal epigenetic programming [11]. The list of chemicals classified as EDCs is constantly growing and includes a wide range of substances used for numerous different commercial or personal applications [12]. Because of their broad distribution in common materials such as paints, plastics, and their abundant use in modern agricultural practices including various types of insecticides, fungicides, and herbicides [13–15], EDCs pose a widespread ubiquitous hazard to human health. Although there have been numerous studies demonstrating that the various classes of EDCs can disrupt many of the body’s endocrine systems [11, 16, 17], there remains little insight into the mechanisms by which EDCs actually induce epimutations at the molecular level and/or the extent of differential susceptibility among individual tissues or cell types to EDC-induced epimutagenesis. Information of this sort would facilitate a greater understanding of the cellular and molecular processes underlying the overall pathological effects of epimutagenesis induced by each specific EDC. EDCs are known to function by disrupting classical endocrine signaling via altering endocrine receptor-mediated signal transduction [18, 19]. Examples of such include EDCs which can interact with the androgen receptor (AR) [20, 21] or estrogen receptor(s) (ERs, including ERα and ERβ) [22]. In addition, however, several reports have described the capacity for certain EDCs to interact with other cellular receptors to alter normal cell function through non-classical endocrine signaling [23]. Thus, EDCs have been shown to interact with orphan nuclear receptors, G-coupled protein receptors, and/or calcium signaling [24–26]. Further, the developmental timing of exposure to an EDC can modulate the extent of resulting epimutagenesis [27]. Specifically, previous research has shown that exposure of fetuses to EDCs in utero can particularly predispose the resulting offspring to de novo induction of disease phenotypes that often then have the potential to be transmitted inter- or transgenerationally [28–30].

In this review, we summarize available information regarding the extent to which direct or indirect exposures of different endocrine systems, organs, tissues, or cell types to various classes of EDCs can result in epimutagenesis. We briefly summarize efforts to determine the effects of EDCs on intact animals in vivo but primarily focus on studies of susceptibility to EDC-induced epimutagenesis among established cell lines maintained in vitro as well as cells maintained ex vivo in primary culture, while comparing the pros and cons of these respective holistic and reductionist approaches, respectively. In addition, we discuss possible mechanisms that may mediate tissue- or cell type-specific, differential susceptibility to different EDCs. Our objective is to integrate available information regarding epimutagenesis induced by different classes of EDCs in different cell types known to express different subsets of relevant endocrine receptors, with that describing the extent to which these EDC exposures can induce epimutations that are then subject to inter- or transgenerational transmission such that subsequent generations are impacted by an initial EDC exposure within a single generation. We suggest that consolidation of this information will have the potential to provide novel insights into mechanistic questions regarding the deleterious effects of exposures to EDCs that have eluded explanation to date. These include (i) how EDC exposure initially induces epimutations at the molecular level, (ii) how EDC-induced epimutations can impact gene expression in the affected cells, (iii) how EDC-induced epimutations initially manifest in one somatic cell type or set of somatic cell types can be transmitted to other somatic or germ cell types within the same individual, and (iv) how germline epimutations can be transmitted inter- or transgenerationally.

Types/Categories of EDCs

As noted above, given their very widespread use in a large variety of products, EDCs are essentially ubiquitous. A list of exemplary EDC-based or EDC-containing products is shown in Table 1, and different categories of EDC-containing products are described below.

Pesticides

EDC-containing pesticides are used for agricultural, commercial, and municipal purposes worldwide to kill unwanted predatory organisms [31]. Common exposures to these chemicals emerge from occupational or environmental exposures via contaminated water, soil, or air [32]. These chemicals can have many damaging effects on the body’s normal endocrine systems facilitated by their ability to bind various hormone receptors in multiple tissues and cell types [33, 34]. Although the harmful effects of dichlorodiphenyltrichloroethane (DDT) use became well-known to the public following publication of Rachel Carson’s book, Silent Spring in 1962, the list of similar chemicals that have been developed and introduced as pesticides continues to grow [33]. An example of a more recent EDC-based pesticide is oxynil (IOX), which is currently used to control a wide range of annual broadleaf weeds [35]. IOX has been shown to lead to significant epigenomic disruptions during thyroid development in gill-head bream (Sparus aurata), and during development of the cardiovascular system in zebrafish [36, 37], as well as other epimutagentic effects discussed below. Additional EDC-based pesticides that have been shown to possess epimutagentic activity include Atrazine [38], Vinlozolin [39], and Methoxychlor [40].

Flame-Retardant Chemicals

Flame retardants comprise a large group of EDC-related chemicals used in a wide array of different consumer products including insulation, electronics, and textiles [41]. Exposure to these chemicals occurs largely through direct contact or inhalation of airborne dust generated as these materials degrade over time [42]. Many different flame-retardant EDC-related chemicals have been phased out or banned over time, including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), due to concern about health risks from rapid bioaccumulation in fatty tissue [42]. However, others have come into use more recently as substitutes such as “alternative” brominated flame retardants and organophosphorus flame retardants [43]. Thus, there remains a need to determine the complete physical, chemical, and epimutagenic properties of individual chemicals in each of these classes in order to optimize their safe use.

Organic Compounds Bisphenol A, Bisphenol F, and Bisphenol F

Bisphenol A (BPA) was originally designed in 1891 by Dianin and synthesized by Zincke in 1905 as a synthetic estrogen, but has been used since the 1950s to generate polycarbonate plastics [84]. Today BPA can be found in polycarbonate plastic containers, thermal receipt paper, and epoxy resins used to line the inside of metal cans and pipes to prevent contamination or
| Chemical Structure | Use | Citation(s) |
|--------------------|-----|-------------|
| Dichlorodiphenyltrichloroethane (DDT) | Insecticide used in agriculture and banned in the USA in 1972. Trade names included Anofex, Cesarex, Chlorophenothane, Dedelo, Dinocide, Didimac, Digmar, ENT 1506, Genitox, Guesapon, Guesarol, Gesarex, Gyprox, Hildit, Ixodex, Kopal, Neocid, OMS 16, Micro DDT 75, Pentachlorin, Rukseal, R50, and Zerdane. Still in use outside of the USA for the control of mosquitoes and the spread of malaria. Human and animal exposure is linked to learning disabilities, breast cancer, and obesity | [44–47] |
| Methoxychlor | Organochlorine pesticide intended to be a replacement for DDT but was banned in the USA in 2003 due to concerns over bioaccumulation toxicity and endocrine disruptor activity. Trade names include Marlate, Chemform, and Methoxy-DDT. Human and animal exposure is linked to learning disabilities, breast cancer, and obesity | [45, 48–50] |
| Atrazine | Currently one of the most widely available herbicides, used for the control of grass and broadleaf weeds. Marketed under the trade names AAtrex and Atranex | [51] |
| Ioxynil (IOX) | Herbicide used for postemergence control of various broadleaf weeds. Marketed under the trade names Actril and Totril | [52] |
| Vinclozolin (VZ) | Dicarboximide fungicide used to control agricultural blights, rots, and molds. Trade names include Ronilan, Curalan, Vorlan, and Touche. Has been shown to have antiandrogenic activity but can also bind to progesterone and estrogen receptors. Exposure in male rats can influence sexual differentiation, reproductive function, and cause transgenerationally maintained kidney and prostate disease | [28, 53] |
| Hexaclorobenzene (HCB) | Used as a fungicide to treat cereal crops to control the fungal disease bunt. Trade names include Anticarie, Ceku C.B., and No Bunt. Has also been used as a wood preservative, synthetic rubber peptizing agent, and for aluminum fluxing and degassing. Human exposure is linked to obesity | [50, 54, 55] |
| Bisphenol A (BPA) | Starting material for synthesis of polycarbonate plastics and epoxy resins. Also found in thermal receipt paper, CDs and DVDs, and inside lining of tin cans. Animal exposure is linked to brain disorders, heart disease, diabetes, and breast or prostate cancer | [56–59] |
| Bisphenol S (BPS) | Used to cure fast drying epoxy glues and as a corrosion inhibitor. Used as a BPA replacement in the production of plastics and epoxies and is now commonly found in many consumer goods | [60] |
| Bisphenol F (BPF) | Used in manufacturing of plastics and epoxy resins. Typically found in tank and pipe linings, industrial floors, structural coatings, and adhesives | [61] |
| Di(2-ethylhexyl) phthalate (DEHP) | Phthalate is used as a plasticizer to make plastics more flexible. Commonly found in a range of industrial and consumer products made with polyvinyl chloride (PVC). Trade names include Bisofex 81, Bisofex DOP, Celluflex DOP, Compound 889, Corfex 400, Diacizer DOP, Eviplast 80, Fleximetl, Flexol DOP, Hercoflex, Kodaflex DOP, Palatinol AH, Plasticizer DOP, Sicol 150, Stafllex DOP, and Truflex DOP. Human exposure is linked to infertility | [45, 62, 63] |
Table 1: (continued)

| Chemical                          | Structure | Use                                                                 | Citation(s) |
|----------------------------------|-----------|----------------------------------------------------------------------|-------------|
| Diethylstilbestrol (DES)          | ![Structure](image1.png) | Nonsteroidal estrogen medication banned in the USA in 1971. Two most commonly used brand names were Stillbestrol and DESPlex. Human exposure is linked to anatomical deformation of the reproductive tract and cancers | [19, 46, 64] |
| Zearalenone (ZEN)                 | ![Structure](image2.png) | Estrogenic metabolite produced by some Fusarium and Gibberella species. Commonly found as a contaminate of cereal crops, such as maize, barley, oats, wheat, rice, and sorghum | [65, 66] |
| Decabromodiphenyl ether-209 (BDE-209) | ![Structure](image3.png) | Polybrominated diphenyl ether (PBDE) congener commonly used in the production of flame-retardant mixtures. Makes up 49.6% of the octa-PBDE marketed under the trade name Bromkal 79-8DE as well as 96.8% and 91.6% of the respective deca-PBDE products which have the trade names Saytex 102E and Bromkal 82-0DE | [42, 67] |
| 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) | ![Structure](image4.png) | PBDE congeners are used in the commercial production of flame-retardant mixtures. BDE-47 makes up between 38.2% and 42.8% of the respective commercially available penta-PBDE products marketed under the trade names DE-71 and Bromkal 70-5DE. Animal exposure is linked to increased risk of obesity | [67, 68] |
| Tetrabromobisphenol A (TBBPA)     | ![Structure](image5.png) | Second most commonly used brominated flame retardant in the USA and Canada behind PBDEs and followed by HBCD. Trade names include Great Lakes BA-59P, Saytex RB-100, Bromdian, Fire Guard 2000, Firemaster BP 4A, and Tetrabrom | [69] |
| Hexabromocyclododecane (HBCD)    | ![Structure](image6.png) | Brominated flame retardant used in polystyrene foams in thermal insulation and electrical equipment. Trade names include Bromkal 73-6CD, Myflam 11645, Pyroguard SR 105, Saytex HBCD, and SR 104. Stereoisomer contaminates can also commonly be found in packaged consumer foods | [70] |
| P-n-nonylphenol (NP)              | ![Structure](image7.png) | Antiandrogen and estrogenic alkylphenol that originates from the degradation of nonylphenol ethoxylates which are used widely as industrial surfactants. Has also been found in soft drinks and dairy products. Animal exposure is linked to brain disorders | [58, 71–73] |
| P-n-octylphenol (OP)              | ![Structure](image8.png) | Estrogenic alkylphenol that originates from degradation of industrial surfactants. Has also been found as a contaminant in water as well as soft drinks and dairy products | [73] |
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) | ![Structure](image9.png) | Highly potent congener of the polychlorinated dibenzodioxins (PCDDs) or simply referred to as “dioxins.” A byproduct of producing certain chlorophenols or chlorophenoxy acid herbicides and burning or organic materials. Human exposure is linked to autoimmune disease, breast cancer, and endometriosis | [45, 46, 74] |
| 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB-153) | ![Structure](image10.png) | Manufactured in the USA between 1930 and 1977 for use in coolants and lubricants in electrical equipment such as capacitors and transformers as well as in pigments, dyes, and carbonless copy paper. Trade names include Aroclor and Chlorectol. Human exposure is linked to infertility, endometriosis, learning disabilities, stroke, and Parkinson’s disease | [45, 63, 75–77] |

continued
Organotins have been used in a variety of applications including as pesticides, herbicides, and as anti-fouling agents added to paints intended to protect boat hulls from the formation of rust. Trade names include Alumacoat, Bioclean, FLoTin, Fungitrol, TinSan, Ultrafresh, and Vikol. Animal exposure is linked to obesity.

**Table 1: (continued)**

| Chemical                     | Structure | Use                                                                 | Citation(s) |
|------------------------------|-----------|----------------------------------------------------------------------|-------------|
| Perfluorinated octyl acid (PFOA) | ![Structure](image1) | Used in a variety of applications since the 1940s primarily as an additive in the manufacturing of fluropolymers which include products such as Teflon® non-stick cookware and Gore-Tex® textiles | [78, 79] |
| Perfluorinated octyl sulfonate (PFOS) | ![Structure](image2) | Used in a wide range of applications. Was the key ingredient in Scotchgard® produced by 3M until phaseout efforts began in 2000 | [78, 79] |
| Tributyltin (TBT)           | ![Structure](image3) | Organotins have been used in a variety of applications including as pesticides, herbicides, and as anti-fouling agents added to paints intended to protect boat hulls from the formation of rust. Trade names include Alumacoat, Bioclean, FLoTin, Fungitrol, TinSan, Ultrafresh, and Vikol. Animal exposure is linked to obesity | [80–83] |

Mycotoxins are secondary metabolites produced by fungi and are capable of causing disease and even death in humans and other mammals [89]. Mycotoxins that result from mycotoxin poison exposure are analogous to the pathologies caused by exposures to pesticides or heavy metal residues [89]. Exposure to mycotoxins in humans and animals often comes from ingesting contaminated foods and feeds stored in hot humid climates that promote growth of these molds [90]. Some of these mycotoxins such as zearalenone (ZEN) which have structural similarity to 17β-estradiol have been classified as EDCs due to their ability to bind to ERs in susceptible mammalian target cell types [65]. Zearalenones are biosynthesized through a polyketide pathway by fungal species of the Fusarium genus which are common contaminants of cereal and maize crops worldwide [66]. Although international guidelines have been established to control contamination by mycotoxins, inadequate implementation of better practices continues to negatively impact human health as well as causing economic losses due to product spoilage caused by these contaminants [91].

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**Estrogen Mimetics—Diethylstilbestrol**

Environmental estrogens are one of the most extensively studied classes of EDCs. A classic example of an estrogen mimetic EDC is diethylstilbestrol (DES). DES was developed in 1938 by Dodds et al. [92] and was found to have steric similarity to estradiol such that it could act as an estrogen mimetic in the body. In the 1940s, DES was used to treat pregnant women in an effort to decrease miscarriages and premature births, which at the time were thought to be due to an imbalance of progesterone and estrogen [93]. In 1953, randomized controlled clinical trials rating the effectiveness of DES found it had no effect on the prevention of miscarriages or premature birth rates [94]. Instead exposure to DES led to development of clear cell carcinoma in 0.1% of the pregnant women who used the drug and caused a decrease in the rate of successful future pregnancies [19]. In addition, female children born from women who took DES during pregnancy developed anatomical malformations of the cervix, vagina, and uterus, while male offspring of DES mothers developed testicular hypoplasia, cryptorchidism, and epididymal cysts [19]. Due to these findings, the U.S. Food and Drug Administration (FDA) advised against using DES in 1971 [19]. Although DES is no longer used for medical purposes, it is still commonly used in research to determine the effects estrogen mimetic EDCs can induce in animal or cell models.

**EDCs Can Function through Classical and Non-Classical Mechanisms**

EDCs possess steric similarity to normal endogenous hormones and can therefore interact with normal intracellular hormone receptors as either agonists or antagonists of classical hormonal
signaling pathways, depending on the particular EDC [18]. Normal endocrine signaling typically relies upon specific hormones interacting with specific cellular receptors to form complexes that then translocate to the nucleus and recruit co-activator proteins to bind to specific DNA response elements that regulate gene expression. Because different endocrine receptors and related signaling pathways are active in different cell types at different stages, EDCs can impact expression of different sets of genes normally regulated by distinct endogenous hormone receptors, thereby eliciting distinct phenotypes [23]. The process of remodeling chromatin to facilitate transcription factor–DNA interactions is largely accomplished by alteration of histone modification and DNA methylation patterns which regulate chromatin accessibility in specific regions of the genome. There have been multiple reports indicating the potential for EDC-induced classical hormone signaling to alter DNA methylation and histone modification patterns via ER-mediated activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway in the Michigan Cancer Foundation-7 (MCF-7) human breast cancer cell line [95, 96]. There has also been evidence that EDCs can alter histone modifications regulating the expression of acetyltransferases potentially altering the activity of the epigenetic machinery via nuclear receptor signaling [97]. In addition to induction of epimutations via interactions of EDCs with classical endocrine receptors and signaling pathways, some EDCs have been reported to interact with other cellular receptors to alter normal cell signaling through non-classical hormonal signaling [98–100]. Examples include EDCs that have been shown to interact with orphan nuclear receptors, G-coupled proteins, or intracellular calcium signaling [24–26]. While there has been considerable lack of evidence indicating the potential for EDC exposure to induce epimutations through non-classical signaling, there was one report of changes in DNA methylation of genes involved in the regulation of cell proliferation (e.g. apoptosis and DNA repair) following exposure of the MCF-10F epithelial breast cell line which lacks expression of both ERα and progesterone (PgR) receptors to BPA, suggesting this EDC has the capacity to function through non-classical hormone signaling [101]. However, the presence or absence of ERβ in the MCF-10F cells used in this study was not assessed, raising the possibility that the observed changes in DNA methylation were actually the result of classical hormonal signaling [102]. Another study utilizing THP-1 human monocytes found that p-nonylphenol (NP) and p-n-octylphenol (OP) exposure suppressed Mdc and IP-10 expression in an ER-independent manner and led to suppression of lipopolysaccharide-induced extracellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (p38-MAPK) signaling pathways [103]. However, it is also possible that NP was functioning through the pregnane X receptor (PXR) [103]. These reports demonstrate the complex challenges facing efforts to identify specific intracellular signaling pathways impacted by exposure to EDCs in general and in response to exposure to any specific EDC in particular.

Developmental Timing of Exposure Modulates EDC-Induced Epimutagenesis

Despite many reports indicating that developmental or postnatal exposure to an EDC is capable of inducing epimutations [104, 105], very little work has been done on the impact of the exact timing of the exposure. This includes both the stage(s) or age(s) at which the exposure occurs and the length of the exposure period. This concept is relevant given the varying effects induced by endogenous hormones at different pre- or postnatal stages or ages [106]. Interestingly, the primary impact of hormones on adult cells is thought to be “activational” because they typically induce transcriptional activation of certain sets of genes in target cells, and the resulting gene expression then ceases when the exposure terminates [107]. In contrast, endocrine effects during development are often termed “organizational” because they can permanently alter the organization (specification, differentiation, proliferation, morphology, and/or function) of cells within developing tissues and organ systems. Disruptions of either of these types of effects can potentially lead to development of disease in the adult [107].

The one study of the window of susceptibility to EDC-based induction of transgenerational epimutations during development was focused on exposure of pregnant rats to the EDC vinclozolin, which led to malformations of sexual development in male offspring [10]. These findings from the Skinner Lab demonstrated that exposure of pregnant female rats (F0 generation) to EDCs prior to and during the fetal period of gonadal sex determination and germline-specific epigenetic reprogramming produced a reduction in the spermatogenic capacity of adult male rats in the ensuing F1–F4 generations, while exposures later during gestation had no effect on adult spermatogenesis in F1–F4 descendants [10, 108, 109]. Further, later studies by the Skinner Lab demonstrated that exposure of pregnant female rats during this established window of susceptibility not only led to the induction of epimutations but also led to an increase in the development of disease states in the offspring which were transgenerationally maintained [10, 28]. It has been suggested that the development of disease states could potentially be the result of EDC-induced epimutations which lead to the establishment of epigenetic programming patterns that are then maintained and transmitted intra-, inter-, or transgenerationally [110–112].

One potential explanation of developmental stage-specific increases in susceptibility to EDC-induced epimutations is that exposures may coincide with periods of epigenetic programing or reprogramming in the embryo or fetus [113]. The two primary windows of epigenetic reprogramming during mammalian development include embryonic reprogramming in the preimplantation embryo and germline-specific reprogramming in the fetus, while periods of epigenetic programing typically precede and/or coincide with differentiation of each somatic tissue or cell type. Embryonic reprogramming has been primarily studied as changes in the methylome (genome-wide DNA methylation pattern) in the preimplantation embryo beginning immediately after fertilization achieved by fusion of the highly specialized sperm and ovocyte epigenomes [114]. Levels of DNA methylation decrease markedly from the zygote through blastocyst stage and reach a nadir termed the naive state in cells of the inner cell mass and trophectoderm of the blastocyst [115, 116]. This DNA-methylation reprogramming process is also coincident with reprogramming of other epigenetic marks primarily histone modifications which reflect a global redistribution of chromatin features [117]. Global DNA methylation patterns are then re-established as the developing embryo undergoes gastrulation and the epiblast is formed, from which the three germ layers (ectoderm, mesoderm, and endoderm) and the germ line are subsequently derived in the mouse [115]. Somatic programing refers to locus-specific changes in DNA methylation in key genomic regions such as at gene promoters or enhancers that establish differential activation or repression of tissue-specific...
genes required for the proper differentiation of distinct cell types [118, 119].

Germline reprogramming is a second genome-wide reprogramming event that occurs uniquely in the developing (fetal and neonatal) germ line [120]. It follows, and is more extensive than, embryonic reprogramming and involves erasure of most DNA methylation including that at imprinted loci which are not subject to reprogramming in the preimplantation embryo. This results in the most hypomethylated state of the epigenome in any cell type at any developmental stage or postnatal age, which has been termed the “epigenetic ground state” [120]. In addition, this second wave of epigenetic reprogramming is coincident with loss of the histone protein H1, “loosening” the DNA, as well as loss of both active (H3K9ac) and repressive (H3K9me3 and H3K27me3) histone modifications indicative of the global euchromatin state the chromatin exists in during this period [121]. The germline DNA demethylation event is then followed by yet another resetting of global and locus-specific DNA methylation patterns that facilitate gene expression patterns required for the sexually dimorphic process of gametogenesis in each sex, and subsequently for early embryogenesis in the next generation [120]. Beyond these reprogramming events, there is one additional period of epigenetic programing/reprogramming that is unique to histone modifications and chromatin structure in spermatogenesis, and which involves replacement of the majority of histones by protamines and extensive condensation of chromatin within the elongating spermatid [122]. This replacement of histones with protamines and concomitant loss of nucleosomes is largely genome-wide, with only a small proportion of the sperm genome (2–15% depending on the species) remaining complexed with histone-containing nucleosomes [123]. The genomic location of a majority of these retained nucleosomes is random, and may simply physically separate domains of protamine-complexed sperm chromatin called toroids [123]. However, the locations of a small proportion of the retained nucleosomes have been shown to coincide with promoters or enhancers that regulate genes that undergo transcriptional activation very shortly after fertilization and encode products necessary for early embryonic development [124]. Elsewhere in the genome, the replacement of histones by protamines facilitates condensation of the genome and concomitant termination of transcription due to extensive condensation of the sperm nucleus during late spermiogenesis. This process is then reversed shortly after fertilization as sperm protamines are replaced with oocyte-supplied histones during subsequent chromatin decondensation of the male pronucleus, while loci which remain complexed with histones during fertilization are epigenetically primed to initiate transcription in the early embryo via this heritable programing [125, 126].

Thus, there are two major waves of widespread epigenetic reprograming that occur during early embryogenesis and fetal germline development, respectively, both of which result in largely decondensed, highly accessible, euchromatic chromatin state [115], that are potentially uniquely susceptible to environmentally induced epimutations such as that associated with EDC exposure [127]. The two additional epigenetic programing events (programing of specific somatic cell lineages and spermatogenesis-associated replacement of histones by protamines) also represent potential developmental periods of enhanced susceptibility to EDC exposure. However, neither of these involves genome-wide chromatin decondensation, so these stages are likely less susceptible than the embryonic and germline reprogramming stages. Evidence reported to date suggests that epimutations induced by exposure to EDCs during germline reprogramming appear to have the highest likelihood of being heritable such that they can be transmitted either intergenerationally (to only one or, at most, two subsequent generations) or transgenerationally (to three or more subsequent generations) [128].

### EDC-Induced Epimutations Can Predispose Disease States

EDCs and the epimutations they induce can impose a significant impact on many biological systems. Exposures to these chemicals can interfere with hormone biosynthesis and/or signaling, metabolism, and numerous other physiological functions in a way that can lead to novel disease states. Diseases that are linked to EDC exposure have been well documented in a number of reviews [45, 129–131]. Examples of diseases that have been shown to be induced by exposure to EDCs include breast cancer [57], brain disorders [58], infertility [63], kidney or prostate disease [45], and metabolic diseases such as obesity [83], among many others.

Studies investigating EDC exposures that have been linked to the formation of cancers have largely focused on those associated with breast cancer due to the large public health impact of this most common cancer in women worldwide. As only 5–10% of breast cancers are due to inheritance of high penetrance genes [132], EDC disruption of epigenetic programing has been identified as a potential additional source of a significant proportion of the remaining cases [15, 46, 47, 133]. It is likely that exposure to EDCs in early life, particularly during breast tissue formation, can alter breast tissue development and increase the incidence of breast cancer in the adult. EDCs linked to the development of breast cancer include estrogen mimetic chemicals, pesticides, dioxins, and solvents, among others [64, 134]. One possible mechanism for the disruption of regulated cell proliferation leading to cancer in the adult breast is EDC exposure in combination with polymorphisms in the gene encoding the biotransformation enzyme, cytochrome p450 1A1 (CYP1A1). This may lead to more rapid metabolism of EDCs or hormone replacement drugs into carcinogenic intermediates, resulting in an increase in the risk of developing postmenopausal breast cancer [135]. Alternatively, perinatal exposure to EDCs may lead to increased sensitivity to progesterone that can also predispose development of breast cancer. Thus, the offspring of mice exposed to BPA in utero and postnatally through milk showed a heightened response to progesterone in the mammary epithelium indicated by over-expression of the Wnt-4 and RANKL genes, leading to more rapid cell proliferation and amplification of other biological responses during the menstrual cycle which were also associated with an elevated breast cancer risk [59]. However, these two examples illustrate only a small subset of the possible mechanisms by which EDCs can promote the formation of breast cancer. Due to the heterogeneity of breast cancers, further research will be required to delineate the full extent to which EDC exposure may enhance the etiology of each different breast cancer subtype.

There have also been extensive efforts by neurobiologists to determine the extent of negative effects exposure to EDCs may have on brain development and neurogenesis [58]. The ability of the brain to respond to hormones begins early during development at the end of the first trimester in humans [136], and during mid-embryonic development in rodents [137]. In the developing brain, steroids can alter neurogenesis, glial development, neural apoptosis, and the formation of synaptic
connections [136, 137]. A key concern is the effect(s) that EDCs can have on the hypothalamic-anterior pituitary axis which functions to integrate peripheral signals and central brain processes involved in the control of adrenal, thyroid, reproductive, growth, and metabolic functions [138]. It has previously been shown that exposure to polychlorinated biphenyls (PCBs) can lead to behavioral impairments including depression [76] and neurodegenerative disorders such as Parkinson’s disease [77].

Additional studies have linked prenatal exposure to pesticides to decreases in neurodevelopment in children, including deficits in motor speed, coordination, visual memory, and visuospatial performance [139], as well as the occurrence of mental disorders such as attention-deficit/hyperactivity disorder [140]. In rat animal models, perinatal or ancestral exposure to the pesticide vinclozolin has been shown to have sex-specific neurobehavioral effects such as decreasing androgen-dependent play behavior in male rats as well as decreasing stress reactivity and increasing anxiety differently in a sexually dimorphic manner between male and female rats [141, 142]. However, due to the complexity of the brain structure and the multitude of different disease states that can occur as a result of EDC exposure, many mechanistic details regarding the manner in which EDCs can negatively impact brain health remain poorly studied.

Finally, there have been many studies that have linked EDC exposure to the development of obesity [50, 83]. This is particularly concerning due to the prevalence of obesity in developed countries and the fact that it represents a significant risk factor for many additional disease states, including type 2 diabetes due to the development of insulin resistance [143]. EDC exposure can alter endogenous hormone balance as well as adipokines such as leptin and adiponectin, affecting metabolism and regulation of energy storage in the body [144]. EDC exposure can also predispose decreased thyroid gland function which can, in turn, lead to weight gain and obesity [145]. In utero exposure to EDCs found in plastics [146], environmentally persistent organic wastes [55], or flame-retardant chemicals [68] have all been shown to lead to increased weight gain and obesity in offspring. An extensively studied EDC to which exposure has been linked with the development of obesity is the organotin TBT [82]. TBT has been shown to activate proadipogenic gene networks in adipose tissue [147], liver [147], and bone marrow [148] through RXR–PPARγ heterodimer signaling. However, more recent reports indicate this signaling is primarily RXR-dependent [97, 149]. Further study will be required to fully understand the mechanism(s) underlying TBT-induced disruptions in endocrine signaling.

**Effects of EDC Exposure on the Epigenome In Vivo**

Although this review is focused primarily on studies of susceptibility to EDC-induced epimutagenesis among cells maintained in vitro or ex vivo, we will briefly summarize past efforts to determine the effects of EDCs on intact animals in vivo to facilitate a comparison of the pros and cons of these reductionist and holistic approaches, respectively. There have been considerable past efforts devoted to the establishment of in vivo models in which to assess the toxicological effects of EDC exposure [150]. Animal models have been heavily used to investigate potential epimutagenic influences and predisposition of disease states within the human population that can result from environmental exposure to EDCs [151]. Indeed, it is on the basis of such whole animal studies that the phenomenon of transgenerational epigenetic inheritance of EDC-induced epimutations and their capability to predispose the subsequent development of de novo adult-onset disease states was originally discovered [10, 152]. A surprising result from these studies was that many EDCs displayed non-monotonic dose–response curves such that they induced deleterious effects following exposure to either low or high doses, while exposure to intermediate doses generated no effect [153]. This initial discovery and subsequent characterization of the dangers of EDCs based on whole animal studies has led to additional efforts designed to identify any tissue-specific epimutagenic effects of exposure to EDCs. A primary example is the multi-phased Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) program which is funded by the NIH and is currently in Phase II [154].

This research consortium was established with the goal of determining the role of environmental exposures in disease pathogenesis as a function of epigenome perturbation while also assessing the utility of surrogate tissue analysis in mouse models of disease-relevant environmental exposures. Programs such as this indicate the continued wide use of animal models to investigate the role that environmental exposures to disruptors such as EDCs can have on disease susceptibility.

While the use of animal models has been quite informative for determining the various disease-related effects associated with exposure to different EDCs, this holistic approach poses certain limitations with respect to investigating the molecular etiology of this phenomenon at the cellular and genomic or epigenomic levels. An immediate experimental variable emerges with respect to the route of administration of the EDC to be tested. Often administration of EDCs to intact animals is done orally via addition of the chemical to the animal’s water supply such that it is provided ad libitum. Alternatively, a test EDC can be administered via injections (e.g. intraperitoneal, intramuscular, intravenous, or subcutaneous) with dose being based on the weight of the animal. Importantly, the route of exposure can significantly affect the bioavailability of the EDC following its introduction. As a result, studies that utilize oral routes of exposure (e.g. gavage) must additionally take into consideration how first pass effects after entering the hepatic portal system will change the bioavailability of an EDC. Thus, something as simple as differences in the route of EDC administration can render comparisons between otherwise analogous studies of the effects of exposure to a particular EDC quite misleading. For instance, distinct metabolic effects associated with different delivery routes can dramatically impact the efficacy of any particular dose or concentration of a test EDC. This is due in part to the complexity of metabolic functions, signaling pathways and distinct cell, tissue, and organ types extant in intact living animal, many or all of which can modulate the effects of an EDC “dose” which can differ depending on the size, organizational makeup, and genetic regulation of metabolism of EDCs within different species. In turn, this can contribute to differences in cell type/stage-specific susceptibility to induction of epimutations.

The complexity of various cell and tissue types further exacerbates the extent of heterogeneity in an intact animal’s response to exposure to an EDC or any other environmental disruptor. Depending on the route of administration of the EDC exposure, certain cell types will very likely be exposed more directly than other types. Yet the range of disease phenotypes that have been reported to be induced by EDC exposures suggests that many more cells or tissues than those that are initially impacted by an EDC exposure ultimately manifest
| Chemical(s) | In vitro/ex vivo cell type | Concentration/length of exposure | Phenotypic effect(s) | Pathway(s)/mechanism(s) | Citation(s) |
|-------------|-----------------------------|----------------------------------|----------------------|-------------------------|-------------|
| BDE-47      | Mouse 3T3-L1 preadipocyte fibroblasts | 3–25 nM/8 days                  | Increased adipocyte differentiation. Decreased methylation at promoter of Pparγ2. Increased expression of Cebpα, Cebpβ, Pparγ2, Slc2a4, Fabp4, G6pc, and Lep mRNAs. | Activation of PPARγ but not RXR leads to decreased promoter methylation of Pparγ2. Increased expression of downstream adipocyte genes and increased adipocyte differentiation. | [162] |
| TRO         | Mouse N2A and human SK-N-AS neuroblastoma cells and mouse 3T3-L1 preadipocyte fibroblasts | N2A and SK-N-AS cells—DES: 10 μM; BPA: 10 μM; TCDD: 10 μM; BDE-47: 10 μM; PCB-153: 10 μM; PFOA: 10 μM; PFOA: 10 μM; HBC: 1 μM; TBT: 0.1 μM /48 h each | DES, BPA, TCDD, BDE-47, PCB-153, and HCB led to global decrease in DNA methylation, in N2A cells but not SK-N-AS cells | Not described | [163] |
| TBT         | TRO and 50 μM TBT led to global decreases in DNA methylation; 80 μM BPA led to global increase in DNA methylation in 3T3-L1 adipocytes. TRO, TBT, BDE-47, DES, PFOA, and PCB-153 exposure promoted adipocyte differentiation. TCDD exposure led to global decrease in adipocyte differentiation. | 0.01–1 nM/24 h | BPA and BPS inhibited cell growth, proliferation, and tube formation in HTR8/SVneo cells. BPA decreased DNA methylation at the promoters of genes involved in cell cycle, proliferation, DNA replication, antioxidant and heat shock proteins, and stress and metabolism in HTR8/SVneo cells, except for Gadd45g and DNA methylation changes lead to downregulation of angiogenesis genes VEGF, PCNA, and ICAM1. No change in DNA methylation at these gene promoters. | [164] |
| Chemical(s) | In vitro/ex vivo cell type | Concentration/length of exposure | Phenotypic effect(s) | Pathway(s)/mechanism(s) | Citation(s) |
|-------------|---------------------------|----------------------------------|----------------------|-------------------------|-------------|
| BPA         | GC-2 mouse spermatocytes  | 20, 40, 80 μM/48 h                | GPX7 genes, which had increased promoter methylation | Global increase in DNA methylation, increased expression of Dmnt1 mRNA, and decreased expression of Dmnt3a and Dmnt3b mRNA | [165] |
| BPA         | Porcine oocytes           | BPA: 200, 250 μM/26 h DEHP: 250 μM, 500 μM, 750 μM, 1 mM, 5 mM/26 h | DEHP—No effect. BPA—global decrease in DNA methylation and significant decrease in the expression of Dmnt3b mRNA | Not described | [166] |
| BPA         | SH-SYSY human neuroblastoma cells | 0.1, 1, 10/48, 96 h | 10 μM BPA treatment led to a significant increase in global DNA methylation and expression of Dmnt1 after 96 h | Increased expression of Dmnt1, leads to an increase in global DNA methylation | [167] |
| BPA         | MCF-10F breast epithelial cells | 1–10 μM/14 days | Increased expression of DNA repair genes and decreased expression of apoptosis genes. Hypermethylation of Bcl2l11, Par6δ, Foxp1, Sfrs11 genes and hypomethylation of Rb promoter and Nup98 genes | Possible interaction with ERβ or a non-classical signaling mechanism | [101] |
| BPS         | MCF-7 breast adenocarcinoma cancer cell line | 10 nM, 100 nM, 1 μM/24 h | Hypermethylation of transposons. Increase in the DNA methylation at tumor suppressor gene promoters. Upregulation of cancer pathology genes involved in the PI3K/AKT pathway | ER activation of PI3K/AKT pathway | [96] |
| ZEN         | Human MCF-7 breast adenocarcinoma cancer cell line and MCF-10F breast epithelial cells | MCF-7: 1, 10, 50 μM/24 h MCF-10F: 0.1, 1, 10 μM/24 h | Significant global DNA methylation increase in MCF-7 cells but not MCF-10F cells. Increased DNNMT1 and MGMT in MCF-7 cells. Upregulation of Gapdh, Igf1, L-fabp, Hk2, Pparγ, ERα, and ERβ genes in MCF-7 cells. | Disruption of energy and metabolism pathways within the cells | [168] |
deleterious effects. This, in turn, implies that defects initially induced in the cells directly exposed to an EDC are subsequently propagated to other cell types or tissues within the body. Fascinating questions remain regarding the cellular and molecular mechanisms by which epimutations are initially induced in cells directly exposed to an EDC and subsequently propagated to cells that were not directly exposed to the EDC. The fact that EDCs are believed to function by virtue of their similarity to normal hormones to disrupt normal endocrine signaling raises the possibility that these signaling pathways are most directly involved in the genesis of EDC-induced epimutations. But, beyond that, no direct mechanism has been established to explain how disrupted endocrine function can lead to the formation of epimutations.

Perhaps the most relevant apparent example of propagation of transgenerational epimutations among different cell types is that between somatic cells and the germ line. It seems very likely that most environmental exposures to EDCs or other disruptive effects initially impact one or more somatic cell types or tissues. Yet for the effects of these exposures to be transmitted transgenerationally requires that these defects are ultimately manifested in the germ line [113]. However, just as the cellular or molecular mechanism(s) by which EDC-induced epimutations are initially established are completely unknown, so too are the cellular or molecular mechanism(s) by which the initial epimutations themselves or some subsequent effect of those initial epimutations are transmitted to other somatic cell types or to the germ line. These are examples of the mechanistic questions that might best be approached in an in vitro or ex vivo cell culture model system rather than in an in vivo, intact animal model system.

In the Reproductive Biology field dogma holds that germ cells lack expression of classical steroid hormone receptors and require indirect signaling by the somatic supporting cells of the reproductive tract to respond to circulating endocrine signals, especially those involving steroid hormones [155]. This dogma would suggest that germ cells may not be susceptible to direct epimutagenesis following EDC exposure and can only be impacted via indirect effects mediated by supporting somatic cells. However, reports suggesting the possibility that EDCs can act through non-classical hormone signaling have sparked significant debate regarding the alternative possibilities that epimutations detected in the germ line following EDC exposure are the result of either direct non-classical hormone signaling or indirect paracrine signaling [156–158]. Once again, these outstanding questions illustrate the potential benefit of utilizing in vitro or ex vivo approaches to perform studies at a level of resolution not possible with the use of intact animal models due to the high degree of complexity that accompanies the latter. In vitro and ex vivo models offer the potential to much more precisely assess the cellular and molecular mechanisms by which any particular EDC at any specified concentration and/or length of exposure generates epimutations, because in vitro and ex vivo approaches facilitate highly focused, high throughput,
| Chemical(s) | In vitro/ex vivo cell type | Concentration/length of exposure | Phenotypic effect(s) | Pathway(s)/mechanism(s) | Citation(s) |
|-------------|---------------------------|---------------------------------|----------------------|------------------------|-------------|
| BPA DEHP    | Porcine oocytes           | BPA: 200, 250 μM/26 h DEHP: 250 μM, 500 μM, 750 μM, 1 mM, 5 mM/26 h | BPA—decreased global H3K4me2 methylation and increased expression of Ash2l, Eed, and Ezh2 DEHP—no effects | Not described | [166] |
| BPA         | SH-SY5Y human neuroblastoma cells | 0.1, 1, 10 μM/96 h | Decrease in histone H3K4me3 and H3K9ac. Increase in expression of G9a, Ezh2, Setd1a, Setd8, Hat1, Sirt1, and Riz1 and decrease in expression of Suv39h1 | BPA leads to an increase of Sirt1 expression and subsequent global decrease in H3K9ac | [167] |
| BPA         | MCF-7 human breast cancer cells | 0–1000 nM/6 h | Increase in expression of cancer-associated gene Hoxb9 and increase in H3K4Me3 and histone acetylation at the promoter region of Hoxb9 | Increase of Hoxb9 expression via ER-dependent mechanism indicated by ChIP enrichment of ERα, MLL3 (regulates H3K4Me3), CBP, and p300 (histone acetyltransferases) at the promoter of Hoxb9 | [178] |
| DES         | MCF-7 breast cancer cell line and ELT3 cell line | MCF-7 and ELT3: 50, 100 nM/15–60 min ELT3: 10, 50, 100 nM/7 or 10 days | Increased activity of EZH2 by phosphorylation of S21 causes a decrease in H3K27me3 in MCF-7 cells | Aberrant ER-mediated PI3K/AKT activation of EZH2 may alter chromatin structure in the developing uterus to reprogram gene expression in a heritable manner | [95] |
| ZEN         | Geminal vesicle (GV) stage mouse oocytes | 10, 50 μM/12 h | Decreases in H3K4me2, H3K9me3, and H4K20me1–3 | Changes in histone methylation are disrupting oogenesis, chromatin configuration/compaction, and cell cycle progression leading to an overall decrease in egg competence | [169] |
| ZEN         | Porcine oocytes           | 5, 10, 30 μM/44 h | 30 μM led to overall increases in H3K4me2, H3K9me3, and H3K27me3. Increased expression of Ash2l, Suv39h2, Setdb1, Ezh2, Eed, and Suz12 | Disrupted H3K4me2, H3K9me3, and H3K27me3 levels cause changes in centromeric heterochromatin domains disrupted interactions between kinetochores and spindle microtubules, disrupted embryonic gene expression, cell lineage segregation, and | [170] |
high resolution studies in a manner that cannot be accomplished using whole-animal models in vivo.

In Vitro/Ex Vivo Studies of Cell Type-Specific Differential Susceptibility to EDC Exposure

Past summaries of the effects EDCs on in vitro and ex vivo cell systems have been limited to a small subset of EDCs [159]. While there are now several established in vitro models that can be used to study the effects of EDCs [160, 161], there remains a need to more comprehensively catalogue recent studies of the epimutagenic effects of a wide range of EDCs on various cell types in vitro or ex vivo to better understand the extent to which an in vitro or ex vivo system can recapitulate epimutagenic effects observed in vivo, and the range of cell type-specific susceptibility to EDC-induced epimutations. Here we have attempted to review past in vitro and ex vivo studies to gain insight into the cellular and molecular mechanisms responsible for initial generation and/or subsequent propagation or transmission of EDC-induced epimutations, as well as to better understand the mechanisms that drive intra-, inter-, or transgenerational transmission of these aberrations. Below we summarize studies of the effects of exposure to various different EDCs on various different parameters of epigenetic programing.

| Chemical(s) | In vitro/ex vivo cell type | Concentration/length of exposure | Phenotypic effect(s) | Pathway(s)/mechanism(s) | Citation(s) |
|-------------|----------------------------|---------------------------------|----------------------|-------------------------|-------------|
| NP OP       | THP-1 human monocytes      | 10 nM–1 μM/1 h                  | Suppressed H4 acetylation at the promoter of IP-10 and Mdc     | Suppressed ERK, JNK, and p38-MAPK pathways in an ER-independent manner. NP may be signaling through PXR or NP and OP are suppressing ERK, JNK, and p38-MAPK pathways through non-classical hormone signaling | [103] |
| NP OP       | Human myeloid dendritic cells (mDCs) | 0.1 nM–0.1 μM/4, 12, 24, and 48 h | Suppressed IL-10 expression and increased TNFA* expression. NP increased H3 acetylation in intergenic regions and increased H3K4me3 at proximal promoter and intronic regions of TNFA | Increased TNFA expression via ER, MKK3/6, and p38-MAPK signaling pathway. Suppressed LPS**-induced IL-10 expression | [180] |
| TBT         | Bone marrow-derived mouse mesenchymal stem cells | 5, 50 nM/48 h                  | Decreased levels of H3K27me3 at genes that regulate adipogenesis, including PPARγ, Cebpα, Klf4, Foxo1, and Irx3 | RXR-dependent suppression of histone modification regulating Ezh2 and activation of Ezh1 and Kdm6b promoted adipogenic commitment | [97] |
| IOX TBBPA   | Xenopus laevis XLS8-TRE-Luc cells | 0.01–1 μM/24 h                 | Reduced T3-induced expression of Thrb and Tih2b and suppressed T3-induced post translational modifications of H3 and RNAPII. TBBPA suppressed T3-induced H3K36me3 | Potential suppression of TH-induced activation of RNAPII transcriptional elongation in TH-responsive genes | [179] |

*TNFA, Tumor necrosis factor alpha; **LPS, Lipopolysaccharide.
DNA Methylation

DNA methylation is one of the best studied and mechanistically well understood epigenetic modifications. It is conserved among most plant, animal, and fungal models [172]. In mammals, DNA methylation typically refers to methylation of the carbon at the fifth position of the cytosine base and is typically restricted to cytosines within 5′-CpG-3′ dinucleotides. It functions in cis as a heritable transcriptional repression mark which the cell uses to suppress retrotransposons, regulate the monoallelic expression of imprinted genes, stabilize X chromosome inactivation in female somatic cells, and, most prevalently, modulate chromatin accessibility in promoter or enhancer regions of developmentally regulated or tissue-specific genes [173]. It is well established that EDC exposure can disrupt normal DNA methylation patterns both in animal models, in vivo, as well as human exposure studies [174, 175]. However, the mechanism(s) by which EDCs initially disrupt DNA methylation patterns remain poorly understood, and even less is known about the extent to which there may be differential susceptibility to EDC-induced DNA methylation changes among different cell types. In vitro and ex vivo cell culture systems offer the potential to conduct precise, well controlled experiments investigating the mechanisms of EDC-induced epimutation at the cellular and molecular levels.

Of the published in vitro and ex vivo studies focused on the effects of EDCs on DNA methylation (summarized in Table 2), many have focused on EDCs that are used in the production of plastics due to their wide use and ubiquitous presence in the environment. These include the plastic monomer BPA and its suggested replacement chemical BPS [60]. These plastic monomers display estrogen mimetic properties and have been suggested to impact many different cell types and tissues within the body. There have been multiple reports regarding the role BPA can play in promoting breast cancer [176]. A recent study indicated that BPS can disrupt methylation of transposons in the breast cancer cell line MCF-7, as well as increasing methylation at promoters leading to further silencing of tumor suppressor genes and thereby exacerbating cancer pathology in these cells [96]. These results corroborate a previous study which indicated that exposure of the human breast epithelial cell lines MCF-10F, which lacks ER alpha and progesterone receptor, to BPA induced changes in DNA methylation and subsequent expression of genes involved in apoptosis and the DNA damage response promoting breast cancer transformation [101].

In addition to breast cancer, it has also been shown that cells within the brain are susceptible to BPA-induced epimutations. One study reported a significant increase in global DNA methylation in SH-SY5Y human neuroblastoma cells following exposure to BPA. This was linked to changes in the transcription of DNA methyltransferases and chromatin modifying genes G9a, EZH2, SETD8, SETD1A, HATI, SIRT1 DNMT1, RIZ1, and SUV39h1 [167]. Mouse neuroblastoma cells showed a similar significant global decrease in DNA methylation following exposure to BPA or to the estrogen mimetic DES indicating these effects are likely concentration/cell type-specific [163]. Other reports have been focused on the extent to which BPA and BPS can disrupt gametogenesis or gestation in males and females that have been exposed. A study focused on exposure to BPA of first trimester trophoblast HTR8/SVneo cells which give rise to placental tissue revealed altered DNA methylation patterns within the promoter regions of genes involved in cell cycle, proliferation, DNA replication, antioxidant and heat shock proteins, stress, and metabolism which could interfere with the normal implantation and subsequent placental development in the early fetus [164]. Finally, in mammalian germ cells, exposure to BPA has been reported to significantly alter not only global DNA methylation in both male and female gametes but also the transcription of epigenetic regulatory factors [165, 166]. Alterations of DNA methylation patterns in either male or female germ line cells afford a possible pathway by which EDC-induced epimutations may be transmitted inter- or transgenerationally to subsequent generations.

In addition to BPA, there have been other estrogenic EDCs for which epimutagenic effects have been studied. For example, studies of ZEN, the estrogenic mycotoxin produced by some Fusarium and Gibberella species, have revealed the estrogenic effects this EDC can have on breast cancer MCF-7 cells and both male and female mammalian gametes. Reports have shown that MCF-7 breast cancer cells, but not MCF10F breast epithelial cells, showed a significant increase in DNA methylation following exposure to ZEN [168]. These effects were thought to be due to the EDC exposure altering expression of energy metabolism genes and those encoding hormone/nuclear receptors (GAPDH, IGF1, L-FABP, HK2, PXR, PPARy, ERα, ERβ), shifting these cells toward a cell state more predisposed to development of cancer pathology [168]. Exposure of either porcine or mouse oocytes to similar concentrations (30 and 50 μM, respectively) of ZEN caused a global increase in the levels of DNA methylation [169, 170]. While there was no change in the levels of Dnmt3a, Dnmt3b, and Dnmt3l mRNA in mouse oocytes, the porcine oocytes did show increased levels of Dnmt3a and Dnmt3b transcripts indicating these mRNAs may resist degradation following changes in global DNA methylation induced by ZEN treatment [169, 170].

Finally, there have been efforts devoted to determining the role EDC-induced DNA methylation changes can have on the development of obesity. Studies in this area have been motivated following results from the Blumberg Lab showing that mice exposed to TBT in utero develop significant changes in chromatin accessibility patterns in F3 and F4 sperm relative to those seen in non-treated controls. Interestingly, these differential patterns of chromatin accessibility showed significant similarity to aberrant patterns of DNA methylation observed in F4 adipose tissue [5]. In vitro studies have shown that mouse 3T3-L1 preadipocytes are susceptible to EDC exposure from BDE-47, BPA, or TBT, which can change DNA methylation patterns globally and at specific adipocyte master regulatory genes promoting adipocyte differentiation [162, 163]. These reports suggest that ancestral exposure to EDCs such as TBT can cause DNA methylation changes that are transmissible through both meiosis and mitosis predisposing animals to accumulate adipose tissue and obesity phenotypes.

Histone Modifications

In addition to causing changes in DNA methylation patterns, EDC-exposures have been shown to disrupt other parameters of epigenomic programming, including patterns of histone modifications. Several different modifications can occur within the highly basic histone amino (N)-terminal tail region [177]. These modifications can influence interactions of these (N)-terminal tails either between subunits of the same nucleosome or among subunits of adjacent nucleosomes. These modifications can also recruit and interact with chromatin remodeling enzymes, which can then change the overall structure and conformation of chromatin. In this way, histone modifications can regulate gene transcription by influencing accessibility of promoter
creased levels of H3K36me3 at the Thibz Thrb and XL58-TRE cells [179]. Exposure to both IOX and the pesticide IOX and the flame-retardant TBBPA altered T3-cations to promote adipocyte lineage commitment [97]. Lastly, a fication regulators such as EZH1, EZH2, and KDM6B, and other exposure increased expression of genes encoding histone modi-

sequences to transcription complexes required to initiate gene expression.

In vitro and ex vivo studies have led to reports describing the widespread effects that EDC exposure can have on histone modifications in many different cell types as shown in Table 3. These effects vary depending on the specific histone modification, including whether each specific mark is a transcriptional activator or repressor. Additional reports have described EDC-induced disruptions of editors of histone modifications, including writers, readers, and erasers. For instance, exposure of SH-SYSY human neuroblastoma cells to BPA led to a significant overall increase in global 5-hydroxymethyl cytosine (5-hmC) prevalence coupled with a significant overall decrease in H3K9me3 and H3K9ac marks which the authors suggested may be linked to changes in expression of genes encoding proteins impacting DNA methylation and chromatin modifications, including G9a, EZH2, SETD8, SETD1A, HAT1, SIRT1 DNMT1, RIZ1, and SUV39H1 [167].

Other studies of breast cancer cell lines showed that MCF-7 cells treated with DES displayed increased expression of the histone methyltransferase, EZH2, which resulted in decreased prevalence of the repressive H3K27me3 mark via ER activation of the PI3K/AKT signaling pathway [95]. Similarly, when human MCF-7 cells were exposed to BPA, there was an increase in expression of the cancer related gene, HOXB9. This increase in HOXB9 transcripts was due to an ER-dependent mechanism that led to elevated levels of H3K4me3 and histone acetylation at the HOXB9 promoter region [178]. Another study showed that mouse mesenchymal stem cells exposed to the organotin TBT were more readily predisposed to differentiate into adipocytes. Specifically, this study showed that TBT acted via RXR-dependent signaling to induce changes in levels of H3K27me3 and H3K4me3 in promoters of genes regulating adipogenesis, including Ppara, Cebpa, Klf4, Foxo1, and Irf3 [97]. In addition, TBT exposure increased expression of genes encoding histone modification regulators such as EZH1, EZH2, and KDM6B, and other acetyltransferases which are known to remodel histone modifications to promote adipocyte lineage commitment [97]. Lastly, a study focused on disruption of thyroid hormone by EDCs found the pesticide IOX and the flame-retardant TBBPA altered T3-induced histone H3 and RNAPII post-transcriptional modifications that are closely linked to transcriptional elongation in Xenopus laevis XLS8-TRE cells [179]. Exposure to both IOX and TBBPA led to reduced capacity of T3 to induce expression of Tlrb and Thibz, and exposure to TBBPA alone also led to decreased levels of H3K36me3 at the Thibz gene [179]. These observations suggest that IOX and TBBPA may suppress TH-induced activation of RNAPII transcriptional elongation in genes directly responsive to TH. Each of these studies showed that EDCs have the ability to modulate histone modifications in different sus-
cceptible cell types in ways that can alter the expression of epige-
netic regulators, which, in turn, can alter expression of a variety of genes normally regulated by the related epigenetic mechanisms.

There have also been reports describing effects EDC expo-
sure has on cells of the immune system via both classical and non-classical signaling pathways. One study showed that expo-
sure to the flame-retardant chemicals NP and OP interfered with the production of chemokines in THP-1 human monocyte cells [103]. This study found that EDC exposure suppressed H4 acetylation at the promoters of two chemokine genes, IP-10 and Mdc, via an ER independent pathway by inhibiting ERK, JNK, and p38-MAPK signaling. Additionally, when human myeloid den-
dritic cells were exposed to NP there was an increase in expression of TNF-α which was correlated with an increase in the level of H3 acetylation in intronic regions of the TNFA gene [180]. This study also detected increased levels of H3K4me3 at the proximal promoter of the TNF3 gene, and in intergenic regions flanking the TNFA gene in NP-treated human myeloid dendritic cells [180]. These effects were correlated with increased nuclear levels of the histone methyl transferases MLL and WDR5 [180]. These changes were ascribed to activity of both ER-mediated and MMK3/6-p38 MAPK signaling pathways [180]. Taken together, these observations show that EDCs can function through both classical and non-classical signaling pathways.

Finally, there have been studies of the effects of EDC-exposure on histone modifications in gametes maintained ex vivo. In one case, exposure of cultured porcine oocytes to BPA resulted in a significant decrease in overall levels of H3K4me2 [166]. In another study, mouse oocytes exposed to ZEN in culture showed changes in levels of H3K4me2, H3K9me3, and H4K20me1, 2, 3, which affected chromatin configuration and compaction, cell cycle progression and oogenesis in general, resulting in decreased overall egg competence [169]. In yet another study, porcine oocytes exposed to ZEN in culture showed increased levels of transcripts encoding the histone methyl-transferases ASH2L, SUV39H2, SETDB1, EZH2, EED, and SUZ12, resulting in increased levels of H3K4me2, H3K9me3, and H4K20me1, 2, 3, indicating that ZEN impacts pathways that regulate the maintenance of normal histone methylation patterns. Therefore, exposure to ZEN could lead to dysregulated gene expression patterns [170]. While high H3K9me3 levels and high mRNA levels of H3K9me3 regulators might alter the centromeric heterochromatin domain disrupting interactions established between spindle microtubules and kinetochores leading to mal-
formed spindles and disorganized chromosomes [170]. These reports again highlight the potential for EDC exposure to induce epimutations in the form of histone modifications which have the potential to be transmitted to subsequent generations via epigenetic inheritance.

Chromatin Structure

Relatively little information has been reported regarding the po-
tential for EDC exposure to directly induce changes in the struc-
ture of chromatin; however, it is very likely that the potential for EDCs to induce disruptions of either DNA methylation or histone modifications patterns, as discussed above, also results in changes in chromatin structure or accessibility. Because DNA methylation and histone modifications are both heritable epige-
netic marks, any disruption of normal chromatin structure in-
duced by either of those marks will also have the potential to be heritable. Studies of EDCs produced as byproducts of industrial detergent production, such as NP, were shown to significantly decrease sperm motility in samples of rat semen [181]. However, this exposure did not adversely affect chromatin integ-

Epimutable Hotspots in the Epigenome

M motif enrichment analysis has been used to screen for specific genomic sequences or sequence characteristics that display
elevated susceptibility to EDC-induced disruptions of DNA methylation patterns. F3 descendants of both pregnant rat and mouse dams treated with vinclozolin displayed alterations of DNA-methylation patterns in the promoter regions of many genes in sperm [30, 182]. In a majority of these promoter regions there was a similar motif termed the “Environmental Induced Differential Methylation Consensus Sequence 1” (EDM1) [30, 182]. However, while this EDM1 motif was associated with 68.8% of differentially methylated regions (DMRs) in sperm, it was associated with only 7.1% of DMRs in somatic Sertoli cells in F3 generation descendants of F0 rat dams treated with vinclozolin [158]. Further analysis has indicated that if there are sequence motifs that increase the susceptibility of promoter regions to EDC-induced DNA methylation changes they are likely cell type- and/or EDC-specific [183].

It has also been suggested that there may be epigenetic control regions (ECRs) within the genome which normally regulate expression of gene clusters, such that disruption of these elements following EDC exposure may lead to dysregulation of multiple associated downstream genes, and this could mediate the formation of cell type-/ECR-specific DMR patterns [158, 184]. ECRs are believed to epigenetically regulate gene clusters ranging from 2 to 5 megabases in size and function in a manner similar to imprinting control regions (ICRs) [185]. It has been shown that fetal exposure can disrupt DNA methylation at the ICR that regulates the maternally imprinted Snrpn gene, and this suggests that EDC exposure could also disrupt other ICRs or ECRs within the genome as well [3]. Future genome-/epigenome-wide analyses will be required to delineate the extent to which, and EDC- or tissue-specificity with which, EDCs may induce epimutations at ICRs or ECRs, and the effects such disruptions may have on gene expression patterns [184].

An additional intriguing possibility is that EDC exposure may also disrupt DNA and/or histone modification patterns at genomic elements that regulate 3D organization of the genome [186]. One study showed that F3 and F4 generation descendants of pregnant mice treated with TBT throughout pregnancy developed abnormalities in the pattern of chromatin accessibility in their sperm in genomic regions that overlapped with regions or “iso-directional differential methylation blocks” (isoDMBs) associated with genes involved in metabolic function in adipose tissue [5]. An interesting characteristic of these specific isoDMBs was that CG-rich regions of the genome were typically hypomethylated, while AT-rich regions were typically hypermethylated, suggesting that sequence-based structural features may mediate differential susceptibility to EDC-induced epimutagenesis.

**Inter- and Transgenerational Inheritance of EDC-Induced Epimutations and Related Disease States**

A particularly alarming feature of the etiology of EDC-exposure based epimutations is the fact that these effects can be transmitted to multiple subsequent generations even in the absence of any further or ongoing exposure to the causative EDC [10, 30, 182]. The etiology of non-communicable disease states that result from exposures during development to extrinsic disruptors such as EDCs is now studied as a subset of the phenomenon termed “developmental origin of health and disease” (DoHAD) [187, 188]. Some of the early examples of this phenomenon came from studies of environmental conditions or parental behaviors that could lead to disease states [189]. A prime example was the effects of prenatal exposure to famine and ensuing health outcomes in children born during the Dutch Famine which occurred between 1944 and 1945. One study showed that developing fetuses exposed to extreme caloric restriction during the first trimester of pregnancy were predisposed to obesity later in life [190]. This and many other related studies established the foundation for development of the “Barker” or “thrifty phenotype” hypothesis proposed in 1990 by the British epidemiologist David Barker, which suggested that environmental conditions associated with low birth weight and premature birth have a causal relationship with the origins of hypertension, coronary heart disease, and non-insulin-dependent diabetes manifesting in middle age [191].

More recent studies have focused on the question of how the epigenome is altered in response to exposures to extrinsic factors and how this can predispose potentially heritable disease states in the individual and his or her descendants [192]. It has been shown that EDCs can induce epimutations which are mitotically or meiotically heritable and can be propagated intergenerationally by cellular proliferation within a single generation or intergenerationally by transmission to F1 or F2 generation offspring, or even transgenerationally to three or more descendant generations, even in the absence of any further direct exposure beyond that which occurred during a single developmental window in the F0 generation pregnant female [10]. Two recent studies examining the mechanism by which EDC-induced epimutations can be transgenerationally transmitted in vivo showed that male F1 descendants of pregnant dams who were exposed to either of two EDCs, DDT or vinclozolin, displayed altered DNA methylation patterns throughout development of the germ line, ranging from fetal to adult ages [193, 194]. This persistence of disrupted DNA methylation patterns throughout germ line development and gametogenesis is consistent with inter- or transgenerational transmission of epimutations following a single initial exposure to an EDC. However, this persistence is, itself, a surprising phenomenon because, as noted above, the processes of embryonic and germline-specific reprogramming of the methylene which both include global demethylation followed by global remethylation [115] would otherwise be expected to erase and correct such epigenetic abnormalities [113], thereby restoring normal “wild type” DNA methylation and histone modification patterns required to direct normal establishment of cell identity and fate as part of each wave of epigenetic reprogramming [195]. Thus, it appears that either a portion of the epimutations induced by exposure to EDCs are able to escape erasure and/or correction during normal reprogramming, or the fidelity of the reprogramming process itself may be diminished by exposure to an EDC in a way that predisposes the stochastic appearance or disappearance of epimutations throughout development of the germ line [8, 152, 196].

One recent encouraging observation noted that DNA methylation epimutations induced by EDC exposure in utero could be corrected by supplementation of the maternal diet with methyl donors such as folic acid [174]. This finding suggests there may indeed be effective ways to mitigate or reverse the deleterious effects of exposures to environmental disruptions such as EDCs simply by promoting consumption of a healthy diet or increasing daily exercise [197–199].
Differential susceptibility to endocrine disruptor-induced epimutagenesis

Conclusions

Although it is well established that environmental exposures to EDCs have the potential to alter the epigenome and predispose disease states, there are limited reports focusing on the differential susceptibility among individual tissues or cell types to EDC-induced epimutagenesis. Further insight into such tissue- or cell type-specific differential susceptibility to EDC-induced epimutagenesis would provide valuable additional insight into the possible mechanism(s) by which EDCs initially induce epimutations at the molecular level as well as the mechanism(s) by which these EDC-induced epimutations can then subsequently impact gene expression potentially leading to the development of disease states. Studies indicating the relative susceptibility of different specific tissues or cell types to EDC-induced epimutagenesis utilizing in vitro methodologies have shown that EDC exposure can alter both DNA methylation and histone modifications to different extents in a variety of different cell types [95, 96, 163, 178]. These reports primarily implicate the involvement of EDC-induced disruption through classical hormone receptor pathways, confirming that many of the body’s hormonally responsive tissues have an increased epimutagenic susceptibility to these environmental exposures. While it has also been suggested that EDCs can function through non-classical hormone signaling [98–100, 103] studies in which this phenomenon has been unequivocally demonstrated have been limited.

Data from in vivo studies have indicated in utero EDC-exposure can induce epimutations throughout the epigenome [30, 200]. This has fueled speculation that initial (primary) EDC-induced epimutations could disrupt normal expression of genes encoding DNA methylation and/or chromatin modifying factors which act as the “epigenetic machinery” that establishes and maintains appropriate epigenetic landscapes within each cell type. Thus, primary epimutations have the potential to predispose additional epimutations throughout the epigenome within cells initially exposed to an EDC. Beyond this, there also appear to be mechanisms—yet to be defined—by which epimutations in the initially exposed cells can be propagated to, or otherwise predispose the genesis of similar epimutations in, other cells that were not directly exposed to the causative EDC. This phenomenon applies to proliferation of EDC-induced epimutations throughout the soma, and from the soma to the germ line. Once present in the germ line, these EDC-induced or – predisposed epimutations have the potential to be transmitted to subsequent generations via inter- or transgenerational epigenetic inheritance. Thus, mechanisms remaining to be elucidated include those responsible for the initial establishment of epimutations at the epigenomic level, as well as those responsible for the intragenerational propagation of the initially induced epimutations throughout the exposed individual and those responsible for subsequent inter- or transgenerational transmission of EDC-induced epimutations, or their deleterious effects, from one generation to the next, thereby facilitating the transgenerational epigenetic inheritance of disease states [152, 167].

A majority of previously published studies of the phenomenon, and underlying mechanisms associated with, EDC exposure during early development have utilized whole-animal models [151]. These studies established evidence for the association of EDC-induced disease states with epimutagenic rather than mutagenic effects and revealed that these effects have the potential to be transmitted to subsequent generations through transgenerational epigenetic inheritance. Indeed, there has now been a myriad of disease states ascribed to the effects of EDC exposure in utero, including breast cancer [57], brain disorders [58], infertility [63], kidney or prostate disease [45], and metabolic diseases such as obesity [83]. In addition, these in vivo studies revealed that effects of these EDC exposures often display a non-monotonic dose–response curve [153]. This was a surprising discovery that added a new level of complexity to the phenomenon of EDC-induced epimutagenesis which may further predispose tissue- or cell type-specific differential susceptibility. However, while these past studies based on analyses performed in intact animal models in vivo have been informative, the results have often been difficult to interpret and replicate, and these studies have incurred high costs, and have required large numbers of animals, and extensive labor and time. In addition, the complexity of biological interactions within the intact animal model compounds the challenge of interpreting, replicating, and/or comparing results—particularly with respect to deducing mechanisms responsible for differential susceptibility to EDC诱导的 epimutagenesis. To overcome these concerns, in vitro and ex vivo methodologies offer a more reductionist approach to more precisely assess questions about differential tissue- or cell type-specific susceptibility to EDCs and the mechanism(s) underlying EDC-induced epimutagenesis with much higher resolution than that afforded by whole-animal models.

However, it is important to mention that in vitro and ex vivo methodologies are not without their own potential drawbacks. The process of culturing cells on tissue culture plastic ex vivo, in addition to exposure to different cell culture media which can often contain undefined xenogeneic components such as serum, can have effects on overall cell morphology, the transcriptome, and/or the epigenome, which can complicate interpretation of the effects of exposure to any specific EDC. Another consideration relevant to modeling EDC exposure in vitro or ex vivo is that the metabolism of EDCs within an intact organism can produce metabolites that can either enhance or reduce the deleterious effects imposed by the EDC. An example is the fact that metabolism of the fungicide vinclozolin produces the primary metabolites M1 and M2, of which, M2 is a more potent inhibitor of AR signaling and an inducer of inhibitory activity via the glucocorticoid and mineralocorticoid receptors than vinclozolin alone [201]. In order to overcome these technical issues researchers will need to utilize rigorous experimental design with multiple controls including analyses of (i) untreated cells ex vivo—maintained under the same conditions as the treated cells and “treated” with carrier without any EDC to provide a comparator that will facilitate identification of EDC-exposure-specific effects in the treated cells ex vivo, and (ii) wherever possible, the corresponding cell type in vivo recovered directly from live animals to provide a comparator that will facilitate identification of any ex vivo system-specific effects. In addition, this will also require prior knowledge and testing of any potential primary metabolites of a specific EDC in order to best model the effects of EDC exposure in vitro and ex vivo.

Many mechanistic questions persist regarding the molecular etiology of EDC-induced epimutagenesis and the subsequent intra-, inter-, or transgenerational propagation of the resulting epimutations and deleterious phenotypic effects they engender. As evidence supporting DoHAD accumulates, the rationale for studies to discover mechanisms at the cellular and molecular levels underlying predisposition of adult-onset diseases by exposures during the fetal period increases. Mechanistic questions include (i) the extent to which there exist cell type-specific differences in susceptibility to initial epimutations following exposure to each particular EDC [30, 182], (ii) whether or not there are epimutagenic hotspots within the epigenome, and (iii)
how epimutations are propagated from initially exposed cells to cells that were not directly exposed to the causative EDC. A long-standing question has involved the manner in which epimutations are able to persist despite the normal waves of epigenome-wide reprogramming that occur in the preimplantation embryo and fetal and neonatal germ line which might otherwise be expected to erase inherited epimutations and reestablish normal epigenetic programming, thereby correcting the epimutations. In vitro and ex vivo model systems will likely be very helpful in pursuing answers to these persistent questions.

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Data Availability

All data included in this review were previously published and so they are publicly available.

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