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ENVIRONMENTAL ENDOCRINE DISRUPTORS

1.1 INTRODUCTION

Many man-made chemicals used in industrial and agricultural applications are now widely dispersed as contaminants in the environment. The original uses of these include as pesticides, plasticizers, antimicrobials, and flame-retardants. These chemicals are typically stable in the environment and most are present at small concentrations. The population is exposed to these chemicals in air, water, food, and also sometimes as ingredients in consumer and personal care products. Some of these chemicals have a significant potential to interfere with normal biological functions and cause adverse health effects. Ubiquitously present in the environment, these chemicals may interfere with our bodies’ complex and carefully regulated hormonal messenger systems by mimicking or antagonizing the actions of the endogenous hormones. These chemicals as a group are referred to as endocrine disruptor chemicals (EDCs). As most synthetic compounds have been present in our biosphere since recently in human and vertebrate evolutionary history, biological evolution has not had enough time to evolve mechanisms against their potential adverse effects.

1.1.1 The Endocrine System

Endocrine system and nervous system constitute the two main regulatory systems in mammalian physiology. The endocrine system regulates biological processes in the body from conception through adulthood, including general growth and the development of the brain and nervous system, the growth and function of the reproductive system, and metabolism and blood-sugar levels. The human endocrine system
Endocrine systems include brain and hypothalamic neuroendocrine systems, pituitary, thyroid, cardiovascular system, mammary gland, adipose tissue, and pancreas; ovary and uterus in females; and testes and prostate in males. All these hormone-sensitive physiological systems are vulnerable to EDCs. Source: Adapted from Diamanti-Kandarakis et al., 2009. Reproduced with permission of Endocrine Society.

is an extensive network of hormone-producing glands comprising hypothalamus, pituitary, thyroid, and organs such as female ovaries, male testes, and pancreas as major constituents (Fig. 1.1). These endocrine glands and organs produce and secrete carefully measured amounts of different types of hormones that perform different functions. Hormones are transported throughout the body via the bloodstream exerting physiological effects on their target cells. The target cells for each hormone are characterized by the presence of certain docking molecules, a class of proteins known as receptors. The interaction between the hormone and its receptor triggers a cascade of biochemical reactions in the target cell that eventually modify the cell’s function or activity.

Hormones act at very low blood concentrations and are characterized by their specificity of action on certain tissues and organs. The timing of the hormonal secretion and delivery is critical and carefully orchestrated to maintain the body’s homeostasis (the body’s ability to maintain itself in the presence of external and internal changes), and to the body’s ability to control and regulate reproduction, development, and/or behavior. Human health depends on a well-functioning endocrine system to regulate the release of certain hormones that are essential for functions
such as metabolism, growth and development, and sleep and mood. Some substances known as endocrine disruptors (EDs) can change the function(s) of this hormonal system increasing the risk of adverse health effects.

1.1.2 Endocrine Disrupting Chemicals (EDCs)

EDCs mostly act as mimetic to natural hormones, but some of the EDCs can antagonize the action or modify the synthesis, metabolism, and transport of the endogenous hormones, producing a range of developmental, reproductive, neurological, immune, or metabolic diseases in humans and wildlife. According to the U.S. Environmental Protection Agency (EPA), EDCs have been described as exogenous agents that interfere with the production, release, transport, metabolism, binding, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock et al., 1996). The European Union and WHO definition proposes an ED as “an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function” (Damstra et al., 2002). There appear to be significant differences between the EPA and European definition, as the EPA merely requires interference with the endocrine system, the European definition explicitly requires in vivo evidence that a substance actually causes harm to the organism. However, these two definitions can be considered complementary, as both indicate that the effects induced by EDs probably involve mechanisms relating in some way to hormonal homeostasis and action (Cravedi et al., 2007). A recent Endocrine Society statement stipulated the ability of a chemical to interfere with hormone action as a clear predictor of adverse outcome, endorsing the EPA definition of EDC that focuses on its ability to interfere with hormone action rather than stipulate adverse outcome (Zoeller et al., 2012). Thus, ED was described in the statement more simply as “an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action” (Zoeller et al., 2012). Earlier, a panel constituted by the National Academy of Sciences, chose to describe such compounds as hormonally active agent (HAA), as it was feared that the language of disruption unjustifiably encourages the notion that any interference or influence on the endocrine system is harmful or “disruptive” (NRC, 1999).

The concept of endocrine disruption, the inappropriate modulation of the endocrine system by dietary and environmental chemicals, as a mode of action for xenobiotic chemicals in animals first burst into prominence with the publication of Our Stolen Future by Theo Colborn, Dianne Dumanoski and John Peterson Myers, which is often credited for garnering major public attention to the concern about the hazards posed by EDCs (Colborn et al., 1996). It brought up the issue of man-made chemicals threatening the reproductive capability and intelligence of future generations of humans and wildlife. She and other authors proposed that many EDCs elicited effects at doses far lower than toxicities caused by other modes of action and thus required special regulation (Colborn et al., 1993; Colborn et al, 1996). Since then, the topic has generated considerable controversy. Much of
this controversy centers on determining what chemicals cause detectable adverse effects at exposure levels typically experienced by humans or animals.

EDCs comprise a broad-class of exogenous substances, many man-made chemicals that are widely dispersed in the environment and compounds that can bind steroid hormone receptors. Some chemicals with endocrine disrupting effects are legacy pollutants, such as pesticides and heavy metals, and many are emerging contaminants (Fig. 1.2). Many of these newer compounds are industrial contaminants, such as phthalates (used in the manufacture of plastics to make it pliable), bisphenol A (BPA; used in plastics to make it harder, clearer, and more resistant to heat stress), alkyl phenols (present in detergents and surfactants), polychlorinated biphenyls (PCBs; formerly used in electrical equipment), dioxins (released from incinerators), organochlorine pesticides and organohalogens (used as flame-retardants), and triazine herbicides (atrazine and simazine). There also are pharmaceuticals purposely designed to have hormonal activity, such as diethylstilbestrol (DES), contraceptive agents, and others that are used in the treatment of diseases such as osteoporosis. These xenobiotic compounds have a wide range of chemical structures but all of them have the capacity to disrupt normal hormonal actions. Even though the intended use of pesticides, plasticizers, antimicrobials, and flame-retardants is beneficial, effects on human health are a global concern. Some naturally occurring EDCs can also be found in plants or fungi, such as the so-called phytoestrogens: genistein, daidzein, or the mycoestrogen zearalenone.

1.1.3 Sources of EDCs in the Environment

EDCs can originate from numerous sources and enter the environment by many routes. From the air, soil, and water, EDCs enter the food chain, and because some of these compounds are lipophilic and persistent, they have the potential to bioaccumulate and become a part of a plant’s or animal’s body burden and biomagnify in higher trophic levels.

Figure 1.2 Grouping of chemicals of some potential endocrine disruptors.
Discharges from municipal wastewater treatment plants (WWTPs) have been identified as significant contributors of EDCs to surface waters (Kolpin et al., 2002; Legler et al., 2002; Snyder et al., 2003). The actual sources are upstream discharges to the treatment facilities, which include natural hormones and pharmaceutical estrogens excreted by humans flushed down home toilets, pharmaceuticals and personal care products (PPCPs) excreted or washed from the body, plant material, items treated with fire retardants, other household cleaning products, and pesticides (Staples et al., 1998; Ying et al., 2002; Snyder et al., 2003). WWTPs might also receive effluents from industrial processes that use cleaners containing nonylphenols and plastics containing BPA or hospital and storm water runoff streams that contain EDCs (Boyd et al., 2004) (Fig. 1.3).

However, WWTP effluents and reclaimed water are not the only sources of EDCs to the environment. Discharges from fish hatcheries and dairy facilities (Kolodziej et al., 2004), fish spawning in natural waters (Kolodziej et al., 2004), runoff from agricultural fields and livestock feeding operations (Orlando et al., 2004; Soto et al., 2004), and land amended with biosolids or manure (Hanselman et al., 2003; Khanal et al., 2006) also contribute as nonpoint sources for EDCs in the aquatic environment. In addition, the potential exists for agricultural runoff containing pesticides and fertilizers to contain the estrogenic surfactants (e.g., nonylphenol ethoxylates) that make up the chemical formulation (Staples et al., 1998; Ying et al., 2002). Other potential sources include private septic systems (Swartz et al., 2006), untreated stormwater flows and urban runoff (Boyd et al., 2004), industrial effluents (Kosaka et al., 2007), landfill leachate (Coors et al., 2003), and atmospheric deposition. Human exposure can occur via the ingestion of food, dust and water, inhalation of gases and particles in the air, and skin contact.

![Figure 1.3](image-url)  
**Figure 1.3** Sources of EDCs in environmental waters.
1.1.4 Deleterious Effects of EDCs on Wildlife and on Humans

Exposure to EDCs in water has been associated with a range of reproductive impacts, particularly in fish, including the induction of intersex (presence of both male and female sex organs) (Jobling et al., 1998), lowered hormone levels (Folmar et al. 1996), and reduced gamete production and fertilization capability (Jobling et al., 2002). WWTP effluents contain a mixture of known or potential EDCs. In most cases researchers have been unable to pinpoint the specific chemicals responsible for effects indicating endocrine disruption in exposed fish. Estradiol, estrone, ethinylestradiol, nonylphenol, octylphenol, alkylphenol ethoxylates, and BPA have been identified as likely causes (Purdom et al., 1994; Damstra et al., 2002) based on their concentrations in wastewater effluents and their potency in laboratory studies.

The adverse effects of EDCs have become an important issue drawing public attention, especially since the link between synthetic birth control pharmaceuticals (e.g., ethinylestradiol) and their toxicological impact on fish was reported (Nash et al., 2004). These concerns have primarily been related to adverse effects observed in wildlife. In wildlife, EDCs are suspected in the decline of certain species (e.g., possible increased sterility in the American alligator), change of sex in fish and shellfish (Vos et al., 2000), eggshell thinning in birds and reptiles, and other problems. As hormone receptor systems function similarly in humans and animals, these observations have raised concern of potential human health effects.

With regard to humans, evidence is limited and inconsistent to clearly establish a causal inference; however, accumulating data is circumstantial evidence linking EDCs to reproductive disorders and disturbed thyroid homeostasis. Key data are the increased incidences of malformations of the reproductive organs in newborn boys, early onset of puberty in girls, as well as increased incidence of certain endocrine-related human diseases. Laboratory studies are correlating the developmental exposure to EDCs with a growing list of adverse health consequences in both males and females. In males, EDCs have been associated with decreases in semen quality/sperm count (Li et al., 2011), testicular germ cell cancer (Chia et al., 2010), and urogenital tract malformation (Fernandez and Olea, 2012). Similarly, EDCs are associated with numerous female reproductive disorders, affecting puberty and breast cancer (Crain et al., 2008; Roy et al., 2009).

Recent research has shown that EDCs also affect physiological systems that control fat development, weight gain, and glucose levels. Endocrine control of glucose homeostasis can impact development of diabetes, obesity, and cardiovascular diseases (Thayer et al., 2012; Newbold, 2010). Some EDCs can transmit health problems across generations, so that exposures during pregnancy can create health problems for several generations. Nonetheless, specific mechanisms by which substances disrupt the endocrine systems are very complex, likely due to time of impact and space being extended, and not yet completely understood.

1.1.5 Endocrine Disruption Endpoints

Endocrine disruption end-effect may be a functional change but is not considered to be toxicological endpoints per se as is cancer or allergy, but more as a mode of
action leading to outcomes such as carcinogenic, reproductive, or developmental effects. These functional changes may or may not lead to an adverse event (Damstra et al., 2002). An adverse effect that is manifested in a physiological outcome in an animal only would be considered as a toxicological endpoint. Primarily reproductive toxicity and impaired development are seen as the well-established endpoints of endocrine disruption. These gross changes observed in vivo can offer presumptive evidence of the toxicity of the chemical or compound under study (CSTEE, 2000).

The three major endocrine disruption endpoints studied are estrogenic (compounds that mimic or block natural estrogens), androgenic (compounds that mimic or block natural testosterone), and thyroidal (compounds with direct and/or indirect impacts on the thyroid) (Snyder et al., 2003). However, EDCs can act via more than one mechanism. Some EDCs have mixed steroidal properties. For example, a single EDC may be both estrogenic and antiandrogenic. EDCs may be broken down or metabolized to generate by-products with different properties. For instance, the estrogen agonist DDT is metabolized into the androgen antagonist DDE (Diamanti-Kandarakis et al., 2009).

Finally, many EDCs may have actions via (or independent of) classic actions at cognate steroid receptors. More recently, studies have shown the activity of the retinoid X receptor (RXR), and peroxisome proliferator-activated receptors (PPARs) to be targets for EDC action. EDCs can affect these systems in several different ways; for example, by directly interfering with receptor signaling or by activating other signaling pathways, in particular that of the aryl hydrocarbon receptor (AhR), a receptor involved in the metabolism of many xenobiotic substances. Thus, all members of the nuclear hormone receptor family are potential targets of EDCs. Chemicals also interfere with metabolism, fat storage, bone development, and the immune system, and this suggests that all endocrine systems can and will be affected by EDCs (UNEP/WHO, 2012).

1.2 SALIENT ASPECTS ABOUT ENDOCRINE DISRUPTION

A number of salient points have emerged defining the specific features of endocrine disruption. While EDCs having effects at high dose and low doses are both of concern, in reality the ones active at low doses are the ones that make it past the typical toxicity screens and are thus of most concern. The EDCs generally occur in the environment as complex chemical mixtures, not single compounds; their impacts can vary substantially over the life cycle of an organism and are often particularly severe during gestation and early development, their impacts can occur long after exposure and many EDCs exhibit transgenerational (epigenetic) impacts.

1.2.1 Low-Dose Effects and Nonmonotonic Dose Responses

It is well established that natural hormones act at extremely low serum concentrations, typically in the picomolar to nanomolar range. Because of shared receptor-mediated mechanisms, EDCs that mimic natural hormones have been proposed to follow the same rules and therefore have biological effects at low doses (Welshons et al., 2003).
Low-dose effects, postulated as typical for EDCs, are defined as biologic changes that occur in the range of human exposures or at doses lower than those typically used in the standard testing paradigm of the U.S. EPA for evaluating reproductive and developmental toxicity (Melnick et al., 2002). Risk assessments for virtually all chemicals, except genotoxic chemicals, assume that, for any substance, there exists a threshold dose below which exposure is safe. However, early studies of EDCs in sensitive animal models have established examples in which no lower threshold dose could be detected; that is, effects were already apparent at the lowest doses tested.

The endocrine system is tuned to respond to very low concentrations of hormone. The typical physiological levels of endogenous hormones are in the range of 10–900 pg/ml for estradiol, 300–10,000 pg/ml for testosterone, and 8–27 pg/ml for thyroid hormone (T4) (Vandenberge et al., 2012). Similarly, EDCs that influence in any way the production, metabolism, uptake, or release of hormones also have effects at low doses, because even small changes in hormone concentration can have biologically important consequences (Welshons et al., 2003). There is also evidence that EDCs work additively or even synergistically with other chemicals and natural hormones in the body (Carpenter et al., 2002). Thus, it is plausible that some of the low-dose effects of an EDC are actually effects of that exogenous chemical plus the effects of endogenous hormone (Vandenberg et al., 2012).

Moreover, there are some EDCs whose effects can be seen at low doses but not at high doses, in opposition to the usual dose–response curve familiar to toxicologists, which shows continually increasing responses with increases in dose (vom Saal et al., 1997). Much like endogenous hormones, which exert their physiological actions through receptors and exhibit nonlinear dose–response relationships, EDCs display a general characteristic of a nonlinear relationship between doses and effect where the slope of the curve changes sign somewhere within the range of doses examined (Vandenberge et al., 2012; EPA, 2013). The dose–response curves can be shaped like an inverted U (Fig. 1.4a), in which low doses increase the response with greatest response at intermediate dose levels, and high doses decrease the response, or like a U with a high response at both low and high levels of exposure (Fig. 1.4b). In some cases the slope of the curve reverses sign at multiple points along the curve (Fig. 1.4c), which could reflect different mechanisms of action at different concentrations. These curves do not conform to the traditional expectations of toxicology, which states that an increase in dose is matched by an increase in effect (Welshons et al., 2003).

Two well-known examples of nonmonotonicity are Lupron used to treat reproductive disorders in women and men and tamoxifen (an ER antagonist) used to treat breast cancer, in which low doses stimulate while high doses inhibit disease. A phenomenon known as low dose “flare” occurs for both of these drugs during which there is stimulation of the response that the drug inhibits when the blood level of the drug is at the high clinically effective dose range (for example, testosterone secretion in men with prostate cancer for Lupron, and proliferation of mammary tissue in women with breast cancer for tamoxifen) (Myers et al., 2009).
Dose–response relationship for individual endocrine toxicants can be an important piece of evidence in the determination of the risk posed by exposure to the toxicant. If some EDCs act directly on receptors, low levels of exposure, particularly during developmentally critical period, can disrupt hormone actions. If the concentration of EDC is higher, it may inhibit receptor-mediated action by down regulation of receptor levels, in which the body reacts to hormonal overstimulation by reducing the number of hormone receptors it produces (Myers et al., 2009). There is also evidence that nonmonotonic dose–response curves are generated by the integration of two or more monotonic dose–response curves that occur through different pathways affecting a common endpoint with opposing effects (Vandenberge et al., 2009; Soto et al., 1995). A 2007 NIEHS-sponsored review of studies of in vivo effects of an estrogenic plastic leachate BPA, for example, identified evidence for effects of low-dose exposure during development on subsequent brain structure, function, and behavior in rats and mice. BPA on low-dose exposure in mice also produces prostate enlargement with an inverted U-shaped dose–response curve (vom Saal et al., 1997).

The endocrine system acts like a thermostat, through self-regulating feedback loops. Receptors typically respond to very low levels of hormone, similarly low levels of an endocrine mimic may activate them, whereas high levels of a chemical may actually cause receptors to shut down altogether, preventing any further response. Very high doses, however, can overwhelm the system and cause damage and even death. It is the body’s responses to BPA at very low doses, operating well under traditional toxicology’s no observed effect level that has been found to result in deleterious effects on mice (Endocrine Disruption Exchange, 2009). The classic example of lead shows that there is no “safe” dose at which no negative effects are found (Lanphear et al., 2005). Thus, traditional toxicological assumptions based on the monotonic dose–response curve, in which more of the chemical leads to a greater effect, may not be applicable to assess the toxicity of EDCs.

1.2.2 Exposures during Periods of Heightened Susceptibility in Critical Life Stages

In cases of endocrine disruption, exposure levels affect organisms during critical organizational periods of early life stages (Guillette et al. 1995). Endocrine
disruption can be profound because of the crucial role hormones play in controlling development (Colborn and Clement, 1992). During development, the genome of the cells that make up tissues and organs become programmed to specify their function in the adult. Because the hormones are naturally present in the body, addition of any hormone-like material from the environment into the body can elicit adverse effects at much lower doses than a toxicant.

There are critical windows of developmental sensitivity to natural or exogenous hormones or hormone-like molecules. In mammals, the late embryonic/early postnatal period is considered a critical period for brain development and sexual differentiation, during which even very short-term exposure to a hormone, or the lack thereof, causes adverse, permanent and irreversible molecular changes in the brain (Gore, 2008). Later in life, in response to increased gonadal steroid hormones during puberty, these organizational effects of hormones on the brain are manifested as appropriate masculine or feminine reproductive physiology and behavior. Adult mammals have feedback mechanisms that can cope with at least some variation in hormone levels. Those mechanisms may not be active during embryonic development, a time at which it is thought animals are most susceptible to the effects of endocrine disrupters. Therefore, EDC exposure during the critical periods of brain development and sexual differentiation is particularly detrimental.

Although hormone signaling is normally reversible and induces dynamic changes in cellular function, during development EDCs can induce permanent effects on gene activity that enable cellular and tissue differentiation. Hormonal effects in the fetus are much more profound because they affect gene expression that governs development of organs as well as lifelong hormonal “set points,” such as receptor numbers and hormonal production.

The exquisite sensitivity of the developing fetus and neonate is suggested to be due to numerous factors including undeveloped DNA repair mechanisms, an immature immune system, lack of detoxifying enzymes, primitive liver metabolism, lack of development of the blood/brain barrier, and an increased metabolic rate (Bern, 1992). Numerous studies exist where age of exposure is a known risk factor. Thus, exposure of an adult to an EDC may have very different consequences from exposure to a developing fetus or infant.

The hormonal environment of the developing fetus is protected from endogenous steroids by conjugation to binding proteins produced by the mother and the placenta. In mammals, steroid-hormone-binding globulins regulate the accessibility of sex-steroids to various organs. For example, bound estradiol is unable to pass the blood brain barrier, whereas unbound testosterone has relatively high access to the brain (Partridge et al. 1980). Little is known about the effects of EDCs on these proteins, or the extent to which they bind. It is therefore not possible to exclude the possibility that some chemicals, because of their particular properties, could more readily gain access to, or accumulate in, the fetus in amounts sufficient to cause effects.

Metabolization of compounds may be faster in children, but detoxification in them is much slow, resulting in greater body burdens due to higher dietary intakes in relation to the body size, compared to adults (Jacobs, 2001). Therefore, at this
stage of life, they are sensitive to changes in the hormonal milieu, or chemical exposure, which can result in organizational changes that are permanent. Even very subtle effects on the endocrine system can result in changes in growth, development, reproduction, or neurologically driven behavior that can affect the organism itself, or the next generation. An exposure to the ED does not need to be chronic, as transient exposure at a critical time during development is all that is required (Damstra et al., 2002).

1.2.3 Delayed Dysfunction

Fetal exposure to EDCs at critical time points, especially during early development when cells are differentiating and tissues are developing, will have harmful health effects that do not become evident until puberty and adulthood.

Animal models consistently demonstrate that low-dose exposures of fetuses to EDCs often have no discernible effects at birth, but result in infertility, abnormalities, and cancers much later in life (Welshons et al., 2006). A compelling example comes from humans, as millions of women who took the estrogenic pharmaceutical DES, under physicians’ advice to avert miscarriage, inadvertently exposed their fetuses to a potent estrogen. The drug had no observable adverse effect on the mother, and at birth, the infant girls appeared externally normal, but later in life they were found to have a disproportionately high level of reproductive-tract abnormality and increased incidence of development of rare vagino-cervical cancers (Newbold, 2004). Laboratory rodent models of DES are quite consistent with the human data, as fetal DES is associated with the latent development of uterine cancer (Newbold et al., 2006).

The implication is that events in prenatal (embryonic and fetal development) and postnatal (infant) stages can affect disease states in adults (Fig. 1.5), also termed as the developmental basis of adult disease (DBAD), leading to the propensity of an individual to develop a disease or dysfunction in later life (Barouki et al., 2012). In other words, the latency between EDC exposure and the emergence of consequential health effects can be considerably long, even decades, and the degree to which gene–environment interactions can produce inter-individual variability is poorly understood. This is especially true for growth and development, processes that are very sensitive to endocrine regulation.

1.2.4 Importance of Mixtures

When it is considered that, in nature, virtually all contamination is in the form of mixtures, the importance of this aspect of endocrine disruption cannot be overestimated. Contamination occurs when the source(s) and nature of the chemicals are man-made. Combinations of EDCs are able to produce significant effect even when each chemical is present at low doses that individually do not induce observable effects. This is true for a variety of endpoints representing a wide range of organizational levels and biological complexity. Some of the contaminants modulate each other’s effects which can be additive, but synergistic interactions
among toxic substances have also been known. These typically occur when two or more compounds contribute to the same endpoint through different mechanisms. A case in point is that of a herbicide S-metolachlor (0.1 ppb) that was found to have no adverse effect on its own in amphibians; however, in combination, harmful effects of atrazine such as retarded larval development and growth were multiplied (Hayes et al., 2006). In practice, these two substances are often mixed together in industrial products.

1.2.5 Transgenerational, Epigenetic Effects
Some EDCs are not only potentially capable of having an effect on the individual but can also transmit health problems across generations. These effects might be transmitted by regulatory factors that control gene expression. This is the case with DES, which caused abnormal female sexual development in the granddaughters of patients who were prescribed the drug. Similarly, an endocrine-disrupting fungicide vinclozolin exposure of pregnant rats resulted in latent development of reproductive dysfunctions, infertility, and cancers in their male F1 offspring (Anway and Skinner, 2006). Moreover, if the F1 males mated prior to the development of disease, their F2 male offspring developed a similar physical attributes, or phenotype. It has been demonstrated that this effect carried at least as far as in the F5 offspring, and that the mechanism involves (at least in part) an epigenetic modification caused by a change in methylation patterns to the male germline (Gore and Crews, 2009). For detailed discussion on epigenetic effects, see Chapter 8.

1.3 HISTORICAL PERSPECTIVE OF ENDOCRINE DISRUPTION
The overall understanding that has been achieved on EDCs is the accumulation of evolutionary and revolutionary steps especially over the last half-century. Some
of the early milestones in environmental hormone research were identified in the e.hormone website of the Tulane University. Recently, a historical perspective on endocrine disruption has also been put together (Marty et al., 2011). Here, we provide a brief history of key events characterizing the development and expansion of the ED hypothesis.

In the 1940s, reports appeared on breeding difficulties in female sheep and cows grazing on pastures rich in red clover (Trifolium pretense) species (Bennetts et al., 1946), which later were found to contain estrogenic compounds such as coumestrol (Adams, 1995). In the 1950s, scientists learnt that certain synthetic chemicals could interfere with the hormones that regulate the body’s most vital systems. This led to the use of steroidal compounds in the livestock industry to modulate reproductive cycles and to enhance rate and efficiency of body weight gain.

In 1962, concerns about DDT in the environment were publicized in Rachel Carson’s book, *Silent Spring*. It described health problems observed in wildlife such as egg-shell thinning, deformities, and population declines linked to pesticides and other synthetic chemicals. Interestingly, Carson intuitively anticipated the ability of certain organochlorines to interfere with reproduction, although at that time the endocrine-disrupting mechanisms were unclear. Studies beginning in the mid-1960s in Lake Michigan suggested that environmental contaminants were adversely affecting hatching success in herring gulls (Keith, 1966). Around 1965–1970, the presence of natural hormones and synthetic estrogens used as birth control agents was noted in wastewater treatment outfalls in the United States (Sumpter, 1995).

The first observation of the impact of EDs in humans was done when DES, a “synthetic estrogen,” was linked to vaginal cancer in daughters whose mothers had taken the drug during the first three months of pregnancy prescribed to prevent miscarriage (Herbst et al., 1971). As a result, the Food and Drug Administration (FDA) advised physicians to stop prescribing DES to pregnant women.

In 1968, about 2000 people were poisoned by hormonally active PCBs and their pyrolysis products polychlorinated dibenzofurans (PCDFs) following consumption of contaminated rice oil in Japan. A similar poisoning by PCBs occurred in Taiwan in 1979 (Aoki, 2001). Later studies on PCB exposure at low–dose levels have found neurodevelopmental and reproductive effects (Brouwer et al., 1999). In 1976, exposure of the population to an accidental release of tetrachlorodibenzo-\(p\)-dioxin (TCDD) from a pressure tank in Seveso, Italy, was found to have various developmental and reproductive effects (Baccarelli et al., 2004; Eskenazi et al., 2000). A similar occupational exposure of Vietnam veterans to TCDD has been associated with an imbalance in thyroid hormone and thyroid-stimulating hormone (TSH) levels (Pavuk et al., 2003).

In 1981, tributyltin (TBT), a constituent of marine antifouling paints, was linked to induction of imposex (penis growth in females) in molluscs, when it was shown that the incidence of the condition was highest close to marinas (Smith, 1981). This became the first well-documented, worldwide population-level effect in wildlife caused by an EDC. Public concern due to adverse effects of environmental chemicals led National Institute of Environmental Health Sciences (NIEHS) to organize conferences on Estrogens in the Environment in 1979 (McLachlan, 1980) and
1985 (McLachlan, 1985). Presentations noted the ubiquitous nature of the contaminants, their potency, and their potential impact on public and environmental health.

In 1991, the idea that xenobiotic chemicals could inappropriately modulate the endocrine system thereby causing detrimental effects in wildlife and humans was first articulated in the watershed first World Wildlife Federation (WWF) Wingspread Conference (Colborn and Clement, 1992). The term endocrine disruptor was coined in this conference. Some plastics widely used in a variety of consumer products were found leaching estrogenic chemicals in laboratory research, mounting the concerns about the potential for environmental chemicals to alter endocrine physiology (Soto et al., 1991). This was the first time that scientists became aware of hormone-altering chemicals in plastics. In 1993, the link between environmental estrogens and male reproductive problems was hypothesized (Sharpe and Shakkebaek, 1993), and associations were drawn between EDCs and declining sperm counts (Toppari et al., 1996; Swan et al., 1997). In 1995, the National Academy of Sciences and National Research Council sponsored a panel study on Hormone Related Toxicants in the Environment (NRC, 1999).

Awareness of endocrine disruption was intensified by the 1996 publication of the book, Our Stolen Future, by Theo Colborn of the World Wildlife Fund and coauthors Dumanoski and Myers, who emphasized on how anthropogenic chemicals have been subtly altering the development, behavior, physiology, and ultimately well being and survival of natural populations, including our own (Colborn et al., 1996). In the same year, a report on male alligators in Florida’s Lake Apopka having strikingly low levels of testosterone and abnormally small phallus size was published. Chlorinated pesticide residues in this contaminated lake due to a chemical spill occurred in 1980 was cited to have caused demasculinization of alligators there (Guillette et al., 1996). Also in 1996, the first known North American report of endocrine disruption in fish below wastewater outfalls was published (Bevans et al. 1996). These reports helped to establish the credibility of endocrine disruption science as a discipline. Since then, a number of important milestones followed.

The first European workshop on the impacts of EDs on human health and wildlife was organized in Weybridge, UK (Weybridge, 1996). Concern about endocrine disruption fostered US legislative mandates including the update of the U.S. Safe Drinking Water Act and Food Quality Protection Act requiring U.S. EPA to implement screening and testing program to detect EDCs (FQPA, 1996). Consequently, EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to evaluate protocols and select a subset to comprise a “Tier I” screening battery for biological activity involving the estrogen, androgen, and thyroid hormone systems. The challenge was made complicated by the need to screen for receptor agonists and antagonists, as well as compounds, which act indirectly, including the inhibition of steroid biosynthesis.

In 1997, BPA, a component of polycarbonate plastic, was shown to alter the reproductive development of lab mice at extremely low doses. BPA mimics the natural sex hormone estrogen (vom Saal et al., 1997). In 1998, widespread occurrence
of intersex fish in British rivers was reported as a consequence of exposure to STP effluents (Jobling et al. 1998). In 1998, Japanese Environmental Agency presented their Strategic Program on Environmental Endocrine Disruptors. In 2000, a book was published reviewing EDCs becoming a social and political concern (Krimsky, 2000). Reports on low-dose effects of EDCs led to National Toxicology Program (NTP) report on EDs low-dose peer review in 2001, concluding that endocrine effects have been demonstrated for a number of chemicals at doses below their previously determined no-effect levels (Melnick et al. 2002). Low levels of atrazine exposure during development were cited for hermaphroditic and demasculinized frogs (Hayes et al., 2002). A USGS study found low concentrations of human and animal drugs, natural and synthetic hormones, detergents, plasticizers, insecticides, and fire retardants in most of the 139 stream sites sampled in 30 states in the United States during 1999–2000 (Kolpin et al., 2002). WHO/International Program on Chemical Safety (IPCS) issued a global assessment of state-of-the-science of EDs (Damstra et al., 2002). In 2002, Terry Collins pioneered introduction of endocrine disruption in his “introduction to green chemistry” class at Carnegie Mellon University (CMU) and from that year he invited annually leading scientists to deliver prominent lectures on the impacts of endocrine disruption on public health. In 2004, long-term exposure to environmental concentrations of ethinylestradiol was reported to cause reproductive failure in zebrafish (Nash et al., 2004). In the following years, endocrine disruption influence on genes’ activity, altering phenotype expression, was recognized to be epigenetic, and transgenerational implications of some EDCs-induced epigenetic alterations were reported (Crews and McLachlan, 2006; Anway and Skinner, 2006; Anway et al., 2005).

In 2009, the Endocrine Society issued a Scientific Statement outlining the scientific evidence supporting the existence and detrimental effects of EDCs and under-scoring them as a significant concern for public health (Diamanti-Kandarakis et al., 2009). In the same year, American Medical Association endorsed this statement and called for new federal policies to decrease the public’s exposure to EDCs. Eight other scientific bodies then joined the Endocrine Society in a letter of concern published in Science in 2011 (Hunt, 2011). In late 2012, a cross-disciplinary team of scientists developed a tiered protocol for endocrine disruption (TiPED) testing to weed out potentially harmful chemicals early in the development (Ritter, 2012). In early 2013, WHO/UNEP published a report on State of the Science of Endocrine Disrupting Chemicals – 2012. It concluded that the speed with which the incidence of endocrine-related diseases increased in recent decades rules out genetic factors as the sole plausible explanation, and points to environmental causes as a contributory factor (WHO/UNEP 2013)). In the summer of 2013, a controversy broke out over the leaked draft proposal of the European Union to regulate EDCs recommending a precautionary approach. The editors of journals of toxicology, endocrinology, and other related fields published combative editorials about how endocrine disrupting chemicals should be regulated (Cressey, 2013).

Table 1.1 shows a summary of various observations of endocrine disrupting effects in wildlife and humans and related progress in understanding their impact.
<table>
<thead>
<tr>
<th>Timeline</th>
<th>Observations</th>
<th>Books/Conferences/Reports/Policy Action</th>
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<tr>
<td>1930s–1940s</td>
<td>Reproductive problems in female sheep and cows grazing on pastures rich in certain clover species, later found to contain estrogenic coumestrol</td>
<td>1950s – Sex steroidal compounds use began for modulation of reproductive cycles and body weight gain in livestock and poultry</td>
</tr>
</tbody>
</table>

Evidence of the health impacts of EDCs grew from the 1960s to the 1990s

Presence of natural hormones and synthetic estrogens used as birth control agents observed in wastewater treatment outfalls in the United States
Exposure of PCBs and PCDFs in populations in Japan (1968) and Taiwan (1979)
Exposure to TCDD in Italy (1976) and Vietnam veterans

1971–1990: DES linked to vaginal cancer in daughters whose mothers had taken the drug during the first three months of pregnancy (1971)
Imposix in molluscs first linked to marine anti-fouling paints containing organotin compound TBT, when it was shown that the incidence of the condition was highest close to marinas (1981)

1991–1995: Some plastic compounds found leaching estrogenic chemicals in laboratory research (1991), made aware of hormone altering chemicals in plastics

Wingspread Conference (1991) proposed first time that xenobiotic chemicals inappropriately modulate the endocrine system causing detrimental effects in wildlife and humans and coined the term endocrine disruptor
<table>
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<tr>
<th>Year</th>
<th>Event</th>
<th>Reference</th>
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<td></td>
<td>NTP report on EDs low-dose peer review (2001), found effects for a number of EDCs at doses below their previously determined no-effect levels USGS study (2002) finds low concentrations of human and animal drugs, natural and synthetic hormones, detergents, plasticizers, insecticides, and fire retardants in most of the 139 stream sites sampled in 30 states WHO/IPCS (2002) issued a global assessment of the state-of-the-science of endocrine disruptors</td>
<td>[2001–2010]</td>
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(continued)
<table>
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<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>2002</td>
<td>Terry Collins pioneers introduction of endocrine disruption in green chemistry class at CMU, and annually invites leading scientists to deliver prominent lectures.</td>
</tr>
<tr>
<td>2007</td>
<td>Introduction of the European legislation REACH</td>
</tr>
<tr>
<td>2009</td>
<td>The Endocrine Society issued a scientific statement outlining the scientific evidence supporting the existence and detrimental effects of EDCs and underscoring them as a significant concern for public health.</td>
</tr>
<tr>
<td>2009</td>
<td>American Medical Association endorses Endocrine Society statement and calls for reduced exposure to EDCs.</td>
</tr>
<tr>
<td>2011–2013</td>
<td>Eight scientific societies in the fields of reproductive biology, endocrinology, reproductive medicine, genetics, and developmental biology in a letter to <em>Science</em> offer expertise for chemical testing and risk assessment.</td>
</tr>
<tr>
<td>2012</td>
<td>A tiered protocol for endocrine disruption (TiPED) testing created for use in the design of new materials to eliminate potentially harmful chemicals early in development.</td>
</tr>
<tr>
<td>2013</td>
<td>WHO/UNEP publish a report on state of science of EDCs.</td>
</tr>
<tr>
<td>2013</td>
<td>Regulation of EDCs gets mired into controversy on leakage of the European Union draft proposal. The editors of journals of toxicology, endocrinology and other related fields published combative editorials about how EDCs should be regulated.</td>
</tr>
</tbody>
</table>
1.4 SCOPE AND LAYOUT OF THIS BOOK

The endocrine disruption science has exploded and has seen an exponential growth in the last two decades. This book summarizes research findings of numerous scientists in several inter-related disciplines. It is not planned as a comprehensive compendium but more of a concise reading for non-specialist audience interested in learning about challenges and possible solutions to endocrine disruption phenomena. The inclusion of key references provides resources for those who wish to go into various topics in more depth.

The book is divided into three parts and 14 chapters. An introductory chapter provides an overview of endocrine system, EDs discusses their salient features and a historical perspective of endocrine disruption phenomena. The first part includes seven chapters. It begins with the second chapter on hormone-signaling mechanisms, followed by laying out various broad classes of putative EDs and a brief introduction to environmental epigenetic modifications. Chapters 3 and 4 describe various putative estrogenic chemicals and heavy metals, and antiandrogenic compounds, respectively. Chapter 5 is devoted to thyroid toxicants while Chapter 6 deals with environmental chemicals that disrupt endocrine system via xenobiotic-sensing and other receptors. In real-world scenario, humans and wildlife are not exposed to one chemical at a time, but rather to complex mixtures. Therefore, Chapter 7 reviews mixture effects of estrogenic, androgenic and thyroid hormone disrupting compounds. The Chapter 8 introduces the emerging science of epigenetic modifications and includes examples of ED compounds with transgenerational effects.

The second part focuses on removal processes of various EDCs by biotic and abiotic transformation/degradation. Chapter 9 includes metabolic and/or microbial biotransformations of ED compounds while Chapter 10 covers various chemical (oxidation) processes for the degradation ED compounds.

The third and final part consists of four chapters, embracing themes on finding solutions to environmental EDCs including their detection, regulation, replacement and remediation. Chapter 11 briefly discusses the endocrine disruptor-screening program of US EPA and various Tier 1 screening assays and includes a discussion on the ongoing efforts for development of high throughput assays. Chapter 12 dwells upon water quality sustainability due to impact of environmental trace contaminants, including EDCs. Chapter 13 addresses policy and regulatory issues relevant to EDCs including scientific uncertainty and precautionary policy. Chapter 14 brings forth the use of Green Chemistry principles in avoiding endocrine disruption in the designing and screening for safer chemicals, and remediation of the EDCs in aquatic environment.

Per evolutionary biologists, ancient estrogen receptor was the precursor of a plethora of other specific hormone receptor systems. The initial identification that...
some of the environmental chemicals affect endocrine system was also related to disruption of endogenous estrogenic activity. Therefore, to date maximum work has been carried out on the activity of various estrogen mimics, including their mechanism(s) of action, low-dose and non-monotonic effects, and adverse impact on health and environment. This would explain, relatively disproportionate space taken in the book by xenoestrogens as compared to other endocrine effects.

The EDCs that have received attention due to their observed effectiveness, abundance or distribution are described in chapters three to six in the book. The reader would keep in mind that hormone actions are pleiotropic (producing more than one effect) — complex endocrine interactions of EDs with other steroids, peptides and lipids are integral to the function of sex-steroids in living vertebrates. Therefore it is critical to consider the enormous range of physiological variation that occurs in vertebrates under “normal” conditions that would be considered “uncontaminated”. Using terms like “estrogenic” or “androgenic” to chemicals may limit the reader because androgens and estrogens have well-characterized function in a very small percentage of species. It is important to note that although compounds may be defined based on a given outcome or mechanism of action, almost all chemicals influence multiple physiological systems and influence the way physiological systems interact with each other.

1.5 CONCLUSION

Pollutants pose destructive consequences to our ecosystem and impose negative health effects to wildlife and humans. It is estimated that about 40% of human deaths (62 million per year) are attributed to the exposure of chemical pollutants (Pimentel et al., 2007). These pollutants include legacy and emerging persistent organic pollutants of chronic toxicity that have been shown or suspected to have endocrine-disrupting properties. Concerns about EDCs have come to the forefront of toxicology only in the last 15 years or so. Traditional toxicological testing had missed endocrine disruption in the first place and overlooked chemicals that could penetrate the womb environment and interfere with the development of the embryo and fetus. The idea that the dose makes the poison (Binswanger and Smith, 2000) has turned out to be overly simplistic. The newest research has clearly shown that biology is affected by low doses of chemicals, and revealed the sensitivity of the developing individual to the slightest chemical perturbation during development.

Recent reports have shown that a number of environmental EDs are capable of interfering with the normal endocrine function in a variety of animals. It has been demonstrated that exposure to a biologically active chemical within the range in which free hormones operate can have an entirely different suite of effects that change during progressive stages of development than when the same chemical is administered in high doses after an individual has fully developed. These studies also confirmed that endocrine effects are time specific, chemical and/or hormone specific, and dose related. Timing of exposure to EDs is found to be of equal or
greater importance than potency, pointing to the importance of the perinatal environment to long-term outcomes of disease states and human health.

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