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Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention

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Abstract
Endocrine Disrupting Chemicals (EDCs) are a global problem for environmental and human health. They are defined as "an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action". It is estimated that there are about 1000 chemicals with endocrine-acting properties. EDCs comprise pesticides, fungicides, industrial chemicals, plasticizers, nonylphenols, metals, pharmaceutical agents and phytoestrogens. Human exposure to EDCs mainly occurs by ingestion and to some extent by inhalation and dermal uptake. Most EDCs are lipophilic and bioaccumulate in the adipose tissue, thus they have a very long half-life in the body. It is difficult to assess the full impact of human exposure to EDCs because adverse effects develop latently and manifest at later ages, and in some people do not present. Timing of exposure is of importance. Developing fetus and neonates are the most vulnerable to endocrine disruption. EDCs may interfere with synthesis, action and metabolism of sex steroid hormones that in turn cause developmental and fertility problems, infertility and hormone-sensitive cancers in women and men. Some EDCs exert obesogenic effects that result in disturbance in energy homeostasis. Interference with hypothalamo-pituitary-thyroid and adrenal axes has also been reported. In this review, potential EDCs, their effects and mechanisms of action, epidemiological studies to analyze their effects on human health, bio-detection and chemical identification methods, difficulties in extrapolating experimental findings and studying endocrine disruptors in humans and recommendations for endocrinologists, individuals and policy makers will be discussed in view of the relevant literature.

Keywords Endocrine disruptors · Exposure · Environment · Pollutants · Estrogenic · Human health

Abbreviations

AR	Androgen Receptor
AHR	Aryl Hydrocarbon Receptor
BPA	Bisphenol A
BMI	Body Mass Index
DDT	Dichlorodiphenyltrichloroethane
EDCs	Endocrine Disrupting Chemicals
EPA	Environmental Protection Agency
ER	Estrogen Receptor
GH	Growth Hormone
HPA	Hypothalamo-Pituitary-Adrenal
ISNT	In Situ Nick Translation
OCPs	Organochlorinated Pesticides
OVX	Ovariectomized
PPAR γ:	Peroxisome Proliferator-Activated Receptor γ
POPs	Persistent Organochlorine Pollutants
PI3K	Phosphatidylinositol 3 Kinase
PCBs	Polychlorinated Biphenyls
COMET	Single Cell Gel Electrophoresis Assay
NIS	Sodium-Iodide Symporter Channel
SCSA	Sperm Chromatin Structure Assay
TUNEL	Terminal Transferase dUTP Nick End Labeling
TCDD	Tetrachlorodibenzo-p-Dioxin
T4	Thyroxine
T3	Triiodothyronine
T2D	Type 2 Diabetes

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1 Endocrine disrupting chemicals: Definition, history and impact on environmental and human health

In early 1990s, a workshop was organized by Theo Colborn and colleagues that introduced the term "endocrine disruptor" and issued a consensus statement (known as Wingspread Statement) about the effects of Endocrine Disrupting Chemicals (EDCs) on human and environmental health [1]. Endocrine disruptors were formally introduced by USA Environmental Protection Agency (EPA) in 1996 as exogenous agents that interfere with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior. Later, the Endocrine Society statement re-defined endocrine disruptors as "an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action" [2]. A wide range of substances, both natural and synthetic, are thought to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, organochlorinated pesticides and plasticizers [3, 4]. Endocrine disruptors may be found in many everyday products– including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides. Research shows that endocrine disruptors may pose the greatest risk during prenatal and early postnatal development when organ and neural systems are forming.

Endocrine disruption is a major public health and environmental health problem. Several environmental contaminants have been reported to have "endocrine disruptor" effects including polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) [3, 5]. These persistent organic pollutants (POPs) cause environmental pollution worldwide and pose several problems for human health. POPs cause carcinogenic, neurotoxic, hepatotoxic, nephrotoxic and immunotoxic effects and birth defects [5–11]. However, in this text, only the endocrine disruptive actions of POPs and other chemicals will be reviewed.

When definition of endocrine disruption was introduced in 1991, researchers mainly focused on estrogenic effects of chemicals polluting the environment [1]. EDCs were therefore initially called xenoestrogens [12]. Several compounds were found to have estrogenic, anti-estrogenic, androgenic and anti-androgenic effects in various *in vivo* and *in vitro* studies [13–16]. Reproductive toxicity of EDCs included reduced fertility in both male and female, breast, endometrial and testicular cancer [17, 18], birth defects of reproductive organs [19], changes in the onset of puberty [20–22] and alteration of the ratio of male to female births [23, 24].

Effects of EDCs on the other endocrine systems have also been reported. Several industrial and environmental chemicals affect thyroid function causing disturbances in metabolism [25–27]. In the recent years, it has been hypothesized that some EDCs (termed obesogens) have adverse effects on insulin action, promote obesity and increase the risk of type II diabetes [11, 28, 29]. EDCs-related metabolic diseases appear to be major risk factors for cardiovascular disease and thus have an important impact on human health. Some POPs alter bone metabolism [8, 30]. Although the mechanisms have not been fully understood, it is likely to be through disrupted hormonal regulation, especially by exerting estrogenic and anti-estrogenic effects on bone formation and turnover processes [8]. A variety of chemicals affect immune system function, hyper-immunity or immune suppression [11]. Thus, there will be an altered response to infection and increased risk of cancer [31]. Effects of EDCs on the immune system are still debated whether it is an "endocrine" effect, however, it is a very important hazardous effect on human health.

In this review, several groups of potential endocrine disrupting chemicals, their mechanism of action as endocrine disruptors, bio-detection and chemical identification methods, epidemiological studies to analyze their effects on human health, difficulties in extrapolating experimental findings and studying endocrine disruptors in humans will be discussed in view of the most recent and relevant literature.

2 Various types of chemicals as endocrine disruptors

There has been an enormous increase in the production of different types of chemicals during the last several decades throughout the World. Although these chemicals have proven to be useful for many aspects of modern life, it is increasingly recognized that they may pollute the environment and cause harm to human beings to varying extent. Of the thousands of manufactured chemicals, it is estimated that about 1000 may have endocrine-acting properties [32].

According to the Stockholm Convention in 2001, production and use of POPs was restricted [33]. Guideline for storage and elimination of a list of chemicals was developed that was appended in 2008 and 2014 [34]. Initially, 12 POPs were identified as "dirty dozen" causing severe adverse effects on humans and the ecosystem. Their production and use were banned. The dirty dozen included pesticides, industrial chemicals and by-products namely aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, PCBs, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.

EDCs comprise pesticides, fungicides, industrial chemicals, plasticizers, nonylphenols, metals, pharmaceutical agents and phytoestrogens. In the next few paragraphs, we will provide brief information about several groups of chemicals that are potential EDCs (Fig. 1). References about

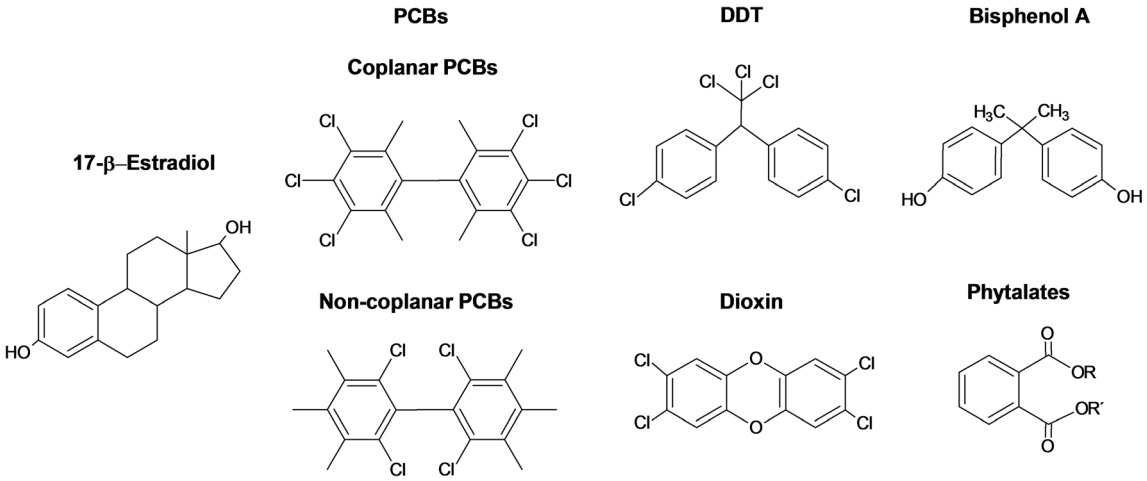

Fig. 1 EDCs comprise various groups of chemicals such as industrial solvents, pesticides, plasticizers and pharmaceutical agents. Structural similarity of some EDCs to E2 is illustrated in the diagram

the properties, manufacturing and use of these chemicals will also be given for further reading.

2.1 Persistent Organochlorine pollutants (POPs)

POPs are chemical substances that may persist a long time in the environment and pose a high degree of toxicity for human health [35]. They are lipophilic substances that can bioaccumulate in the food change, particularly in fatty tissues. Thus, these chemicals may ultimately reach concentrations that have harmful effects on human health and the environment. In addition, POPs can potentially be transported over long distances and dispersed around the World by air and ocean currents and pose not just a local, but global toxic threat. Therefore, appropriate measures were recommended to be implemented by the Stockholm Convention to prevent the release of POPs into the environment or at least reduce such contamination as far as is technically feasible and economically acceptable [33, 34]. Despite the regulations and implementations, POPs are still routinely detected in environmental and human samples.

Polychlorinated biphenyls PCBs are mixtures of different congeners having various numbers (1–10) and positions (ortho, meta, para) of chlorine atoms around biphenyl rings [7, 36, 37]. They were widely used as insulators in capacitors and transformers because of their inflammability and insulating properties, as hydraulic fluids, and in paints and related products. Because of their lipophilic nature and persistent properties, they remain global contaminants in both the environment and the human body [37] in spite of the fact that their production was banned in most countries in the late 1970s [7]. PCBs with biphenyl rings substituted with zero chlorines in the ortho position that assume a coplanar biphenyl orientation in solution, and PCBs with one to four chlorines in the ortho position that assume noncoplanar biphenyl orientation [7]. Several PCB congeners and mixtures have been reported to have neurotoxic, carcinogenic, immunotoxic, hepatatoxic, nephrotoxic and cytotoxic effects in various experimental models and also in human studies [8, 9, 36–41]. With regard to endocrine disruptive effects, noncoplanar PCBs structurally resemble to estradiol 17-β (E2) and hence can mimic estrogenic effects in the cell [31]. In contrast, coplanar PCBs mimic dioxin in that they bind with relatively high affinity to the aryl hydrocarbon receptor (AHR) [42, 43]. It regulates the transcription of a large group of dioxin-responsive genes and results in a reduction in cytosolic estrogen level (i.e. anti-estrogenic effect).

Organochlorine pesticides (OCPs) Pesticides are used as insecticides, herbicides, fungicides and rodenticides. Based on their chemical structure, there five major classes of pesticides; organochlorines (such as DDT, endosulfan, lindane, mirex, dieldrin, chlordane), organophosphates (such as malathion, parathion), carbamates (such as carbaryl, carbofuran, aminocarb), phyrethroids (such as cypermethrin, permethrin) and triazines (atrazine is the most studied triazine herbicide). Although all classes of pesticides may produce adverse effects on human health [44–47], OCPs pose the greatest risk as EDCs because of their lipophilic feature and thus bioaccumulation potential [4]. OCPs are among the persistent organic and environmental pollutants. Occupational and residential exposure to pesticides is a significant problem and subject of several epidemiological studies. Primary exposure of populations to pesticides occurs through food intake and ingestion of these chemicals. However, inhalation and dermal absorption of OCPs (such as DDT) may be important routes of exposure [48].

Endocrine disruptive effects of OCPs have been extensively studied. Their effects on male and female fertility as a consequence of estrogenic, anti-estrogenic, androgenic and anti-androgenic actions, obesity and metabolic disorders, thyroid homeostasis, hormone-sensitive cancers and hypothalamo-

pituitary axis have been reported [4, 43, 49, 50]. Some OCPs structurally resemble to E_2 and thus can bind to estrogen receptor (ER) and mimic the effects of the endogenous ligand. Some xenohormones may display anti-estrogenic actions by binding to AHR and initiating transcription of cytochrome p450 enzymes that metabolize E_2. Residues of OCPs have been found in human tissues all over the World. There are numerous epidemiological studies reporting OCP levels in biological samples (such as serum, fat tissue, breast milk) and investigating relationships to various diseases and disorders in humans [49, 51–54]. Their residues have also been detected in cord blood [50].

Plasticizers & nonyphenols Bisphenol A (BPA) and phthalates are plasticizers that provide shape and flexibility to plastic products. These chemicals may leach out of the plastic material (especially when containing warm food & drinks) into food chain and cause toxic effects, especially in children and pregnant women. Unlike POPs, plasticizers are not lipophilic and do not bioaccumulate in the body. More than 90% of BPA is eliminated in urine as metabolites usually in less than 24 h [55, 56]. However, these chemicals are widely used and there is extensive exposure to them on a daily basis. Over the last decade, some limitations have been implemented about the use of BPA in children's products and thermal paper receipts [57]. Alternative plasticizers have been developed such as Bisphenol S, Bisphenol B and Bisphenol AF [58]. Since these alternative bisphenols are structurally similar to BPA, they are suspected to have equivalent toxicological effects. Various endocrine disruptive effects of plasticizers are reviewed later in the text.

Nonylphenols are organic chemicals with nine carbons bound to a phenol ring. They are commonly used in pesticides, industrial oils and detergents, and thus cause widespread contamination in soil, sediments, water and food [59]. Although information on the effects of nonylphenols on reproductive functions is limited, a review of the recent literature is reported [46].

Heavy metals Populations are exposed to various heavy metals through different routes such as dietary supplements, cigarettes, alcoholic drinks and contaminated air, water and food [46, 60]. Humans are generally exposed to arsenic via ingestion of contaminated food and water. Lead exposure occurs through inhalation of combusted petroleum products, ingestion of drinking water contaminated by lead used in pipes and inhalation/ingestion of particles of lead -based paints. Mercury is another heavy metal that can contaminate water, soil and air. The most common way of exposure to mercury occurs through ingestion of contaminated fish. In a study conducted in Alaska, mercury and arsenic were found in lipid-rich food samples [61]. These findings were taken to suggest that organometals may exert endocrine disruptive effects, especially on reproductive functions. Heavy metals have reported to induce testicular damage. In this regard, cadmium toxicity as environmental contaminant has long been known [62]. Presence of heavy metals as EDCs in human biological samples has recently been reported in a Belgian population [63]. Although there are several reports in the literature [46, 60, 62, 63], the effects of heavy metals on endocrine functions need to be further elucidated.

3 Human exposure to EDCs

Humans are exposed to a broad range of chemicals through different routes. Transmission of environmental pollutants to the human organism occurs usually in an unconscious manner during the daily course of life [35]. Human exposure to EDCs mainly occurs by ingestion and to some extent by inhalation and dermal uptake [43].

Dietary intake is the main entering route of POPs and other EDCs to the human body and accounts for more than 90% of the total chemical exposure [35, 64, 65]. It is well known that organic chemicals, particularly POPs are lipophilic substances and therefore can bioaccumulate in the fatty tissue in the organism. Once these chemicals are spread in the environment, they easily contaminate fish, meat, dairy and poultry products and finally transported to human organism by ingestion. POPs can be stored in the adipose tissue of the body for a long time and thus cause a prolonged exposure to these pollutants. Plasticizers such as bisphenol A do not bioaccumulate in the body with an estimated half-life of about 6 h [66, 67]. They are then excreted into urine. Plasticizers are very often detected in populations, for instance bisphenol A was detected in 92.6% of persons ≥6 years of age in the US [68]. Although plasticizers are not stored in the adipose tissue, there is continuous exposure to these chemicals on a daily basis that raises concern about their endocrine disruptive effects [67].

Some environmental chemicals may be volatilized and transferred to humans through inhalation route [7, 69]. Inhalation may be a very important route of exposure, especially for some volatile chemicals and semi-volatile compounds [35]. Environmental pollutants are often detected in animals such as polar bears living in Arctic areas [70]. Presence of EDCs in the tissues of mammals in remote areas is taken to implicate that these chemicals are long-range dispersed through air as well as ocean currents [7, 71].

Indoor area is an important environment for potential exposure to air-borne chemicals and particles. Occupational exposure to EDCs is also a significant route of contamination for people working at risky environment [35].

As mentioned earlier, POPs are fat-soluble substances, therefore they can be transferred to human body through dermal absorption [7]. Exposure to indoor organic chemicals through the dermal route accounts for only a small percentage

of the total exposure, but can be significant [35]. The risk of transdermal exposure to POPs may be substantially greater at instances like handling contaminated materials or swimming in polluted waters [72, 73].

4 EDCs and female reproductive functions (estrogenic and anti-estrogenic effects)

The female reproductive functions are regulated by the hypothalamus and anterior pituitary gland by secretion of luteinizing hormone and follicle stimulating hormone. These gonadotropic hormones in turn regulate ovarian and uterine functions including synthesis and secretion of gonadal hormones [74, 75]. Since the definition and introduction of xenoestrogens in early 1990s, particular focus has been given to the adverse effects of EDCs to the female and male reproductive systems. EDCs appear to have the potential to interfere with reproductive system by adversely affecting the structure and/or function of female reproductive organs [43]. Disrupted hormone action, irregular cyclicity, decreased fertility, infertility, polycystic ovarian syndrome, endometriosis, hormone sensitive cancer, precocious or delayed puberty and adverse birth outcomes have been associated with some EDCs [46, 76, 77].

EDCs exhibit their toxic effects by interfering with physiology of hormones that promote growth and development of reproductive tissues. These exogenous chemicals interfere with binding of hormones to their corresponding receptors such as ER (Fig. 2) and androgen receptor (AR) that can result in an agonistic or antagonistic effect [78]. An organochlorine pesticide (methoxychlor) has been reported to cause estrogenic action by binding to ERα and ERβ subtypes [79]. Methoxychlor was also shown to bind to AR and cause an opposite effect [80]. A very toxic environmental polluting dioxin, tetrachlorodibenzo-p-dioxin (TCDD) is known to cause antiandrogenic effect [43]. An insecticide, endosulfan also demonstrated estrogenic action *in vitro* and caused ovarian regression by interfering with the hypothalamo-pituitary-gonadal axis [81]. BPA has been reported to bind ERα and ERβ receptors and exhibit estrogenic activity [82, 83]. Breast, ovarian and endometrial cancer incidences are reportedly increasing, and it is suggested that EDCs and other environmental factors contribute to these hormone-sensitive malignancies in women.

In addition to hormone receptor interference, EDCs can also act on enzymes involved in steroidogenesis and metabolism. Phthalates exert anti-androgenic activity by inhibiting testosterone synthesis in Leydig cells [84]. It has also been reported that some EDCs inhibit 5-α reductase, an enzyme that converts testosterone to a very potent androgen, dihydrotestosterone [85]. It should also be noted that EDCs may affect hormone receptor expression. It has been reported that BPA may alter the epigenome and cause dysregulation of steroid receptors [86].

Dioxins and several OCPs can bind to AHR that promotes induction of CYP1 genes expression. They in turn metabolize intracellular E2 and thus cause anti-estrogenic action [43]. TCDD has been shown to interfere with steroidogenesis in the ovaries and decrease steroid levels [87]. A similar anti-estrogenic effect has been reported for metabolite of methoxychlor [88]. Phthalates have also been shown to stimulate AHR and induce expression of CYP1B1 [89].

Adverse effects of EDCs on female reproductive system involves interruption of folliculogenesis [78]. BPA has been suggested to decrease fertility. It triggers apoptosis through a caspase 3-mediated mechanism in the rat follicles and regress luteal development [90]. BPA levels have been associated with decreased number of antral follicles and oocytes in women receiving fertility treatment [91]. This plasticizer has also been associated with polycystic ovarian syndrome, endometriosis, decreased fertilization and risk of implantation failure [92, 93]. Premature births, miscarriages, developmental abnormalities and behavioral deficits have been linked with BPA, phthalates and phenols in human [94–96].

There is strong epidemiological evidence to suggest a relationship between developmental exposure to OCPs and subsequent fertility problems in women [78]. Exposure to EDCs during critical phase of development (pre- and peri-natal period of life) is considered to affect the reproductive system greater than in the adult life. Thus, timing of exposure to EDCs is of great importance in terms of type and severity of adverse effects on the reproductive functions.

5 EDCs and male reproductive functions (androgenic and anti-androgenic effects)

Male reproductive system is also hormonally regulated by the hypothalamus and anterior pituitary hormones, and androgens provide the driving force development of the genitalia [43, 97, 98]. EDCs can seriously affect male reproduction and several studies have reported a decrease in sperm count, reduced motility and abnormal sperm morphology [76, 99]. Birth defects such as cryptorchidism and hypospadias, prostate and testicular cancer have been linked with environmental pollutants. These adverse effects of EDCs on the male reproductive functions have been increasing significantly over the last decades [78, 100].

Sperm count and semen quality have been reported to be low in the male population in the North Europe, particularly in Danish men [101, 102]. In various occupational exposure and environmental contamination studies, reduced male fertility has been attributed to EDCs. For instance, occupational exposure to pesticides brought about abnormal sperm morphology, decreased sperm count and motility [51]. Adverse effects of

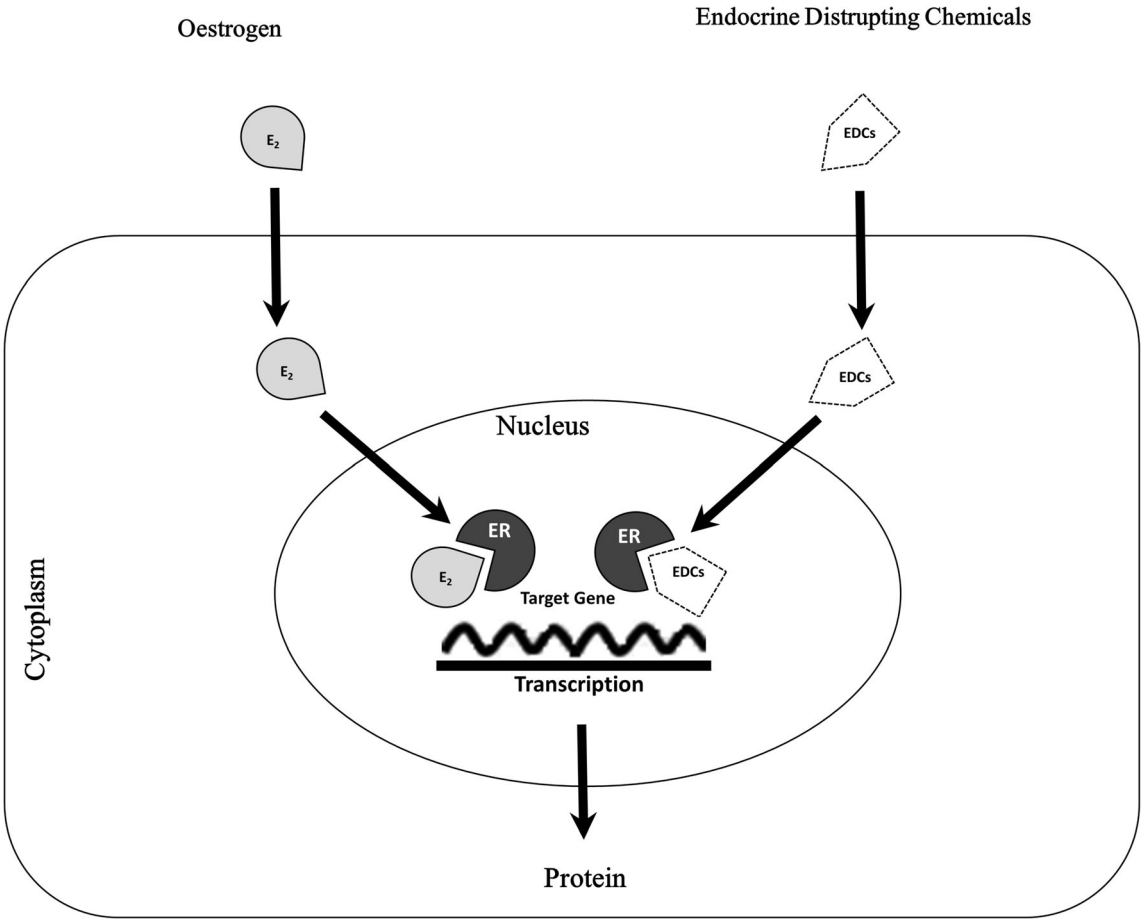

Fig. 2 Schematic representation of mechanism of estrogenic action by E2 and EDCs

OCPs such as DDT and endosulfan have been reported to disrupt hypothalamo-pituitary-testicular axis and interfere with steroidogenesis in the gonadal tissue [52]. Disruption of testosterone by Leydig cells in young Faroese men have been associated with elevated serum PCB levels [103]. Phthalates and BPA exposures have also been reported to have adverse effects sperm count and motility, DNA damage in spermatozoa and testosterone levels in human populations [104, 105]. Exposure to heavy metals such as lead and cadmium were also shown to affect male reproductive parameters [106]. It has been suggested that exposure to EDCs may result in reduced semen quality, testicular cancer, cryptorchidism and hypospadias [107]. It has been shown that neonatal rats exposed to BPA had increased risk of prostate hyperplasia later in life [108].

There is increasing mechanistic evidence that androgenic and anti-androgenic chemicals play important role in impairment in development and maintenance of male reproductive health [43]. Mechanisms that are responsible for the adverse effects of EDCs on male genital development and reproductive functions involve inhibition of 5α-reductase and aromatase [78]. It has been suggested that the sex ratio at birth can be modified by environmental pollutants [109]. Indeed, recent evidence has shown that the Y/X ratio of live spermatozoa was significantly lower in sperm treated with EDCs such as TCDD than control spermatozoa [24]. These findings implicate that a reduction in Y sperm viability may result in a female-biased sex ratio of offspring at birth.

6 EDCs and obesity

Prevalence of obesity and obesity-related diseases are progressively increasing and now at an epidemic level [110]. The endocrine system plays a fundamental role in regulation of energy metabolism. The main cause of metabolic diseases is generally believed to be genetic along with environmental factors such as excessive food intake and lack of physical activity [111]. In addition to these classical factors, it has been suggested that there may be other players in the pathogenesis of obesity to account for the current trend [112]. In the recent years, it has been hypothesized that EDCs stimulate adipogenesis and cause obesity. These EDCs were hence termed "obesogens" [29, 113].

Obesogens may promote adipogenesis and lipid accumulation (Fig. 3). Disruption of lipid homeostasis may involve

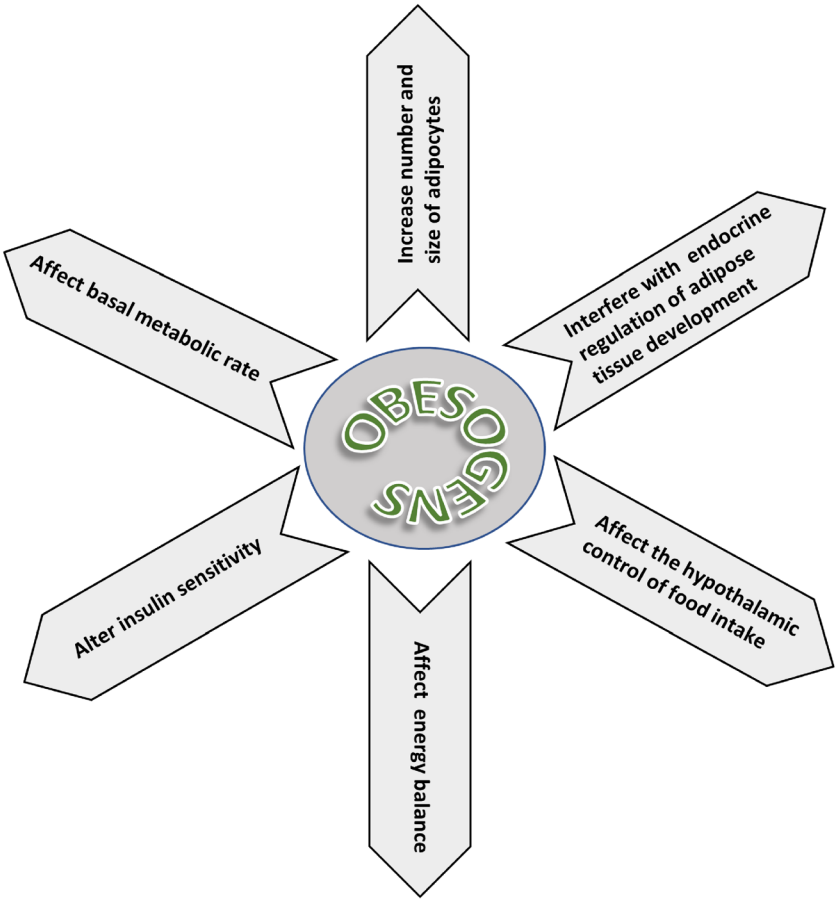

Fig. 3 Obesogens' mechanisms of action in promoting adipogenesis and lipid accumulation

multiple mechanisms [29]: 1) increasing the number of adipocytes, 2) increasing the size of adipocytes, 3) altering endocrine regulation of adipose tissue development, 4) changing hypothalamic regulation of appetite, satiety and food preference, 5) affecting basal metabolic rate and 6) energy balance in favor of calorie storage and 7) altering insulin sensitivity in the liver, skeletal muscle, pancreas, gastrointestinal system and the brain. At cellular level, obesogens can exert their endocrine disruptive effects by interfering with peroxisome proliferator-activated receptors (PPARs) and steroid receptors [29, 114, 115]. These nuclear transcriptional regulators can bind their response elements in the DNA and control gene expression. PPARs can bind to unsaturated fatty acids and thus act as lipid sensors [116]. They regulate lipid influx, adipocyte proliferation and/or differentiation. Some EDCs can bind to these nuclear receptors, alter gene expression that results in obesity.

It has been shown that various EDCs alter adipogenesis through interfering with PPAR-γ function [114]. Tributyltin stimulates preadipocytes to differentiate into adipocytes in 3 T3-L1 cell line, promotes adiposity and impairs metabolic functions in rat by increasing PPAR-γ [117]. BPA, nonyphenol and triflumizole have also been reported to increase adipogenesis [118, 119]. Another plasticizer phthalate also activates PPAR-γ and promotes differentiation of 3 T3-L1 cells into adipocytes [120]. In human studies, detection of phthalate metabolites has been associated with increased Body Mass Index (BMI) and waist circumference [121, 122]. Exposure to POPs is correlated with obesity and disturbance in metabolism [123]. Biologically significant level of exposure to PCBs can promote obesity [4]. In NHANES survey between 1999 and 2002, a significant relationship was found between serum levels of PCB congeners and OCPs with increased waist circumference [124]. In two recent studies conducted in Swedish cohorts, serum levels of OCPs have been associated with altered lipid metabolism and development of obesity [54, 125].

Steroid hormones can also affect lipid homeostasis and hence fat deposition [126]. Adipocytes are known to contain the cytochrome P450 enzyme aromatase (encoded by CYP19A1 gene) which converts androgens to estrogen. Several EDCs have been reported to alter intracellular aromatase activity [127] that result in increased levels of estrogen and corresponding obesity in men [128]. AHR is a ligand-activated transcription factor, usually activated by presence of environmental toxicants such as POPs. Activation of AHR leads to increased expression of xenobiotic-metabolizing enzymes, cytochrome P450s [129]. AHR

pathway may also indirectly affect adipogenesis by changing PPAR-γ expression. Some POPs have been suggested to exert their obesogenic activity thorough this mechanism [130, 131].

Obesogens may also affect the hypothalamic control of food intake and energy balance [132]. There are relatively very few studies directly examining the effects of EDCs on hypothalamic circuits that regulate feeding and satiety. A very recent experimental study has reported that chronic treatment with tributyltin causes changes in orexigenic and anorexigenic hypothalamic systems in a gender dependent manner [133]. Serum BPA levels have been correlated positively with leptin and adiponectin, but negatively with ghrelin hormones in humans [134]. Leptin is secreted by the adipose tissue and inhibits appetite, while ghrelin is produced by the stomach and being stimulatory. Although evidence is limited, it is thought that some EDCs may interfere with the hormonal regulation of hunger and satiety.

Many EDCs (especially POPs) are lipophilic and known to bioaccumulate in body fat over the years. As our recent unpublished data have indicated, the higher is the BMI, the greater will be the retention of EDCs in the fat. Therefore, the relationship of obesity to diseases is not just to the deposition of fat, but to prolonged exposure to POPs. Since several compounds are present in adipose tissue, the body is exposed to a mixture of obesogenic chemicals, rather than being exposed to a single molecule. Therefore, it is likely that they may exert a combination of effects as detailed above. The mixture of chemicals may stimulate adipogenesis at lower concentrations than needed for each compound alone. Timing of exposure to the obesogenic EDCs is another critical issue. Obesogen exposure during pre- and early post-natal life can increase number of adipocytes permanently into adult life [135, 136]. Therefore, early-life exposure to obesogens may cause a prolonged burden of pollutants, especially POPs.

Although over consumption of food coupled with lack of exercise is the major contributor to the increased incidences of obesity, exposure to obesogens may also promote adipogenesis and lipid accumulation. Preventive measures to avoid and/or reduce exposure to EDCs will be discussed later in the text.

7 EDCs and risk for type 2 diabetes

Diabetes is a chronic and complex metabolic disease that arises from persistent insulin resistance and/or insufficient insulin secretion due to β-cell dysfunction or death [137, 138]. Type 2 diabetes (T2D) is a multifactorial syndrome in which genetic predisposition and environmental factors play important roles in its physiopathology [139]. There is growing evidence for a link between EDCs and development of diabetes and related metabolic disorders. Exposure to several environmental pollutants have been correlated with risk of diabetes. These toxicants include PCBs, OCPs, dioxins, bisphenol A and phthalates [140]. Serum PCB levels were significantly associated with diabetes in women included in the Anniston Community Health Survey [141]. In 1979, about 2000 people were accidentally exposed to PCBs and dibenzofurans due to ingestion of contaminated cooking oil in Taiwan [142]. This poisoning event is called Yucheng, "oil-syndrome" in Chinese. It has been reported that women affected during the Yucheng toxic exposure had an increased risk of developing diabetes [142]. This risk was markedly higher in individuals having chloracne, a cutaneous manifestation of dioxin-like PCB exposure, in the same study. In a meta-analysis that included data from six prospective studies showed that POPs are associated with increasing incidence of diabetes [143]. Eleven years after this study, a second meta-analysis was conducted under the name of Nurses' Health Study II and suggest that prolonged exposure to POPs have diabetogenic potential [144]. Similarly, significant relationship has been reported between serum levels of dioxins, PCBs, pesticides and bisphenol A and prevalent diabetes in a systematic review and meta-analysis [145]. Phthalates levels showed a borderline significance with T2D in the same study. Vietnam veterans exposed to dioxins had an increased risk of developing diabetes [146, 147]. Evidence for diabetogenic role for plasticizers bisphenol A and phthalates and POPs such as PCBs, perfluorooctanoic acid, perfluoro octane sulfonate and DDT as well as some heavy metals including cadmium and arsenic has been reported [43, 139, 148].

There are also experimental studies about a possible link between EDCs and diabetes and related metabolic syndrome. Perinatal exposure to an organochlorine pesticide, DDT caused impairment in glucose tolerance (12) and reduced insulin secretion (13) later in adult mice. Similarly, administration of bisphenol A to mice in utero resulted in disruption in glucose tolerance and insulin secretion [149]. Chronic exposure to bisphenol A also impaired glucose metabolism in mice [150].

Metabolic disturbance related to environmental pollutants has usually been attributed to obesogenic effects of EDCs. However, there may be direct effects of these chemicals leading to interference with glucose metabolism and finally T2D as discussed in several epidemiological and experimental studies, above. However, mechanism(s) of EDCs leading to T2D are currently not well documented. It is conceivable that these chemicals (at least some of them) may cause toxicity to pancreatic β-cells [148, 151]. This toxicity may be direct or by induction of reactive oxygen species that increases rate of β-cell death [152]. Recent evidence has suggested another mechanism in which impairment of mitochondrial dynamics has been related to insulin resistance and T2D [153]. Changes in mitochondrial fission and fusion contribute to mitochondrial dysfunction, oxidative stress and β-cell apoptosis that may cause disrupted glucose homeostasis [139, 154]. Administration of tributyltin and chlorpyrifos *in vitro* on

human induced pluripotent stem cells has resulted in decreased expression of mitochondrial fusion protein mitofusin 1 and mitochondrial fragmentation [155]. In summary, EDCs may impair glucose metabolism, insulin secretion and sensitivity either directly or indirectly by acting as obesogens. It should also be noted that genetic predisposition, lifestyle factors such as lack of exercise and weight gain and bioaccumulation of POPS in human body may all contribute to increased risk of developing T2D.

8 EDCs and adrenal hormones

The hypothalamo-pituitary-adrenal (HPA) axis is essential for proper functionality of human body and is a common target for many drugs and environmental chemicals [156]. There are relatively few studies examining cellular mechanisms underlying the effects of EDCs on the adrenal gland and hormones [157, 158]. Most of the reports available have focused on interference with biosynthesis and metabolism of steroid hormones [159]. Various steps in the adrenocortical steroidogenic pathway (hydroxysteroid dehydrogenases, 5-α reductase, steroidogenic acute regulatory and several Cytochromes P450 such as aromatase) may be disrupted by exogenous chemicals [156]. Zone-specific expression of steroidogenic enzymes promote biosynthesis of aldosterone in the Zona Glomerulosa, cortisol in the Zona Fasciculata and adrenal androgens in the Zona Reticulata [157]. It has previously been shown that hexachlorobenzene impairs adrenal steroidogenesis and decreases corticosterone levels in ovariectomized rats [160]. A recent study has investigated prenatal exposure to organochlorinated chemicals on steroid hormones in a Japanese cohort [161]. A total of 29 OCPs were detected in maternal and cord blood that were inversely correlated with cortisol and adrenal androgen levels.

When evaluating adverse effects of ECDs on HPA axis, it should be considered that even a partial interference with adrenal endocrine function may result in severe disturbance in physiological parameters in human. As most of the EDCs bioaccumulate in adipose tissue, usually an exposure model to mixtures of chemicals develops [162]. Therefore, clinical manifestations may appear only after several years of low dose, but constant, exposure to EDCs. As mentioned above, there is limited number of studies examining the effects of EDCs on the HPA axis and adrenal function. More attention has been given to the analysis of obesogens and their effects on the metabolism. Today, comprehensive epidemiological studies are needed to elucidate any relationship between concentrations of various EDCs in human samples and adrenocortical functions / disorders, in addition to mechanistic experiments using *in vivo* and *in vitro* models.

9 EDCs and thyroid hormones

Thyroid hormones are essential for normal brain development and regulation of metabolism, and interference with thyroid function has several consequences at all age levels, particularly during development [163]. Several weeks supply of triiodothyronine (T3) and thyroxine (T4) hormones are stored in the thyroid gland. These hormones are transported to target tissues usually in combination with thyroid hormone-binding globulin, transthyretin or albumin. T4 is converted to T3 primarily in the liver and kidney, and T3 is the active form of thyroid hormones in the target tissues [164].

EDCs may interfere with the thyroid function at different levels [165, 166]. Disruption of thyroid function may involve central regulatory mechanisms at the hypothalamic and/or anterior pituitary level and synthesis, transportation, bioavailability or metabolism of T3 and T4 hormones [167]. Some POPs may inhibit thyroxine 5-deiodinase activity that is responsible for conversion of T4 to T3 [168]. Some environmental chemicals may interfere with thyroid hormone binding to its receptors and hence disrupt signaling directly. Several EDCs have been reported to alter iodine absorption by inhibiting the sodium-iodide symporter channel (NIS), a transmembrane protein responsible for iodide uptake into the thyroid gland [158, 165]. Perchlorate, thiocyanate and nitrate competitively inhibit the NIS and affect thyroid hormone production and bioavailability [169]. Hence, a lower iodine bioavailability may result in hypothyroidism. Perchlorate, thiocyanate and nitrate are widely used in industrial products, but many food and water samples are also contaminated with these EDCs [43]. Several studies by Braverman and colleagues have shown that subjects exposed to perchlorate, thiocyanate and nitrates had low levels of free thyroxine levels [170–172]. This observation was more dramatic in the pubertal subjects.

It is generally believed that PCB exposure decreases serum T4 concentrations in both adult and neonate rodents [173, 174]. One of the mechanisms for this decrease may be the ability of PCBs to induce hepatic microsomal enzymes leading to biliary excretion and elimination of the thyroid hormones [174, 175]. In an early study, Collins and Capen (1980) reported that perinatal rats exposed in utero and by the milk to PCBs showed structural changes in the thyroid gland and reduced serum thyroid hormone levels [176]. Similar observations have been reported by a number of other researchers [177]. However, increased levels of thyroid hormones as an immediate response to PCB administration have also been documented. Six weeks administration with commercial mixtures of PCBs caused an increase in serum thyroid hormone levels in female rats [25]. It has been suggested that structural similarity between thyroid hormones (particularly T4) and some PCB congeners [178]. As a consequence, these contaminants may interfere with thyroid function by binding

to the thyroid transporting proteins, reducing production or increasing metabolism of T4 or reducing conversion of T4 to T3 [173]. It may cause a functional hypothyroidism, most likely due to displacement of the endogenous hormone from the transporting proteins which promote excretion of the thyroid hormone.

EDCs may also cause thyroid cancer [179]. A recent study has provided evidence for linking occupational exposure to biocides and risk of developing thyroid cancer [53]. This population-based case-control study was conducted in Connecticut and involved 462 incident thyroid cancer cases who were occupationally exposed to biocides and pesticides through a job-exposure matrix.

Since thyroid hormones are essential for growth and development, exposure to EDCs during fetal and neonatal life could cause potential adverse effects both on the thyroid function and also on the nervous system. Developmental exposure is critical since thyroid dysfunction is known to cause severe impairment in neurogenesis [43]. Experimental studies have shown that prenatal EDC exposure interferes with thyroid function that in turn causes cognitive and behavioral impairments [180]. Epidemiological studies have also shown that perinatal exposure to thyroid disrupting chemicals cause cognitive deficit and a decline in IQ score in the affected children [165, 181]. A recent study on Danish women revealed that POPs were present in placental samples [27]. Concentrations of these chemicals were inversely associated with thyroid hormones. These findings are taken to implicate that disruption of thyroid hormones may have significant developmental effects on the new born.

10 Environmental Pollution & Growth Hormone Disruption

Acromegaly is a rare endocrine disease that occurs in less than 125 persons in about one million population [182]. It is caused by growth hormone (GH) secreting anterior pituitary tumors. There are relatively very few reports about a possible connection between acromegaly and environmental pollution. The first clinical study was reported by Cannavo and colleagues in 2010 [183]. They evaluated acromegaly patients in about 600.000 population living in four low to high industrially dense areas in the province of Messina, Italy. An increased prevalence of the disease was found in the highly polluted area. This effect could not be explained on the basis of a familial susceptibility or a known genetic predisposition, rather, environmental factors were attributed in the pathogenesis of GH-secreting pituitary adenomas. It has recently been reported that long-term exposure to some POPs increased GH synthesis in an *in vitro* model of GH3 cells, and this finding was associated with increased expression of AHR [184]. AHR pathway has an important role in cellular detoxification mechanisms and tumorigenesis [185]. In a recent study from the Cannavo and colleagues [186], AHR mutation was shown in 9 of 23 acromegaly patients living in highly polluted area in Italy. The functional abnormality of the AHR gene was associated with more severe acromegaly, increased pituitary tumor size and resistance to somatostatin analog treatment [187]. These studies suggest that EDCs may indeed cause alterations in GH secretion, however, understanding of the mechanism(s) requires further experimental and clinical investigation.

11 Testing EDCs, *in vivo* and *in vitro* models

In this section, *in vivo* and *in vitro* test models for analysis of effects of EDCs on various endocrine axis and organs/tissues will be briefly reviewed. Test protocols, their use and sensitivity will be referred. In addition, technology for cell-based systems and genetic models for screening of EDCs in biological, dietary and environmental samples will be discussed.

***In vivo* estrogenicity assay** Uterotrophic assay is an internationally recognized short-term screening method to determine estrogenic and anti-estrogenic properties of chemicals *in vivo* [188, 189]. It is based on the development of uterine tissue under the influence of estrogen. There are two models of uterotrophic assay in which immature and ovariectomized (OVX) young adult rodents are used. In both models, the hypothalamo-pituitary-ovarian axis is not functional so that sensitivity and response of the uterine tissue to exogenous substances can be evaluated. In the immature uterotrophic assay, pre-pubertal (22 day old) female mice or rats are used. In the adult model, young mature rodents are OVX, and adequate time is allowed for uterine tissue to regress. The test substance is administered daily by oral gavage or subcutaneous injection for three days, and then weight of uterine tissue is determined and histomorphometric analysis is performed [13].

12 Vitellogenin assay for xenoestrogens

Expression of vitellogenin in male fish is widely used as a biomarker of exposure to environmental estrogens due to its specificity and sensitivity [190]. Vitellogenin in fish is an estrogen-induced yolk precursor protein. Vitellogenin is considered to be a hallmark of female protein due to its estrogen-dependent synthesis [191]. Its levels in male fish are quite low, and non-physiological induction of vitellogenin in the male is taken to indicate estrogen mediated endocrine disruption. Vitellogenin assay is a well standardized and sensitive endocrine disruptor assay, and there are universal guidelines for this test [190, 192].

In vitro estrogenicity & anti-estrogenicity assays: There are several *in vitro* models to test estrogenic and anti-estrogenic actions of EDCs.

MCF-7 Focus Assay is a human breast-cancer cell assay [193, 194]. In this test, MCF-7 cells proliferate in response to oestrogen and chemicals with estrogenic action. Confluent cells are stained with a fluorescence dye and then staining density is analysed.

Yeast Estrogen Screen Assay utilizes a recombinant yeast strain *Saccharomyces cerevisiae* BJ1991 [195]. Yeast hosts an integrated gene coding for human ER in its genome and expression plasmids carrying the reporter gene lac-Z. When lac-Z gene is activated by presence of estrogenic compounds, β-galactosidase degrades chlorophenol red β-D-galactopyranoside substrate [196].

Luciferase Reporter Gene Assay is an advanced molecular biology method that can be used as a screening test for estrogenic and anti-estrogenic compounds. Luciferase reporter gene assays monitor the transcription of specific genes in cells through detection of light generated by the enzyme luciferase [197]. For estrogenic reporter gene assay, MCF-7 cells are stably transfected and used [198]. For anti-estrogenicity assay, mouse hepatoma cells were stably transfected and used [199]. Dioxin like substances bind to AHR and activate cytochrome enzymes that in turn decreases intracellular concentrations of estrogen. This method is recognized by the European Union as a screening test for analysis of EDCs for dioxin-like activity in biological samples as well as food and environmental samples [200].

13 *In vivo* androgenic assay: Hershberger assay

The Hershberger Assay is a short-term *in vivo* screening assay intended to identify substances with androgenic and antiandrogenic activity [201]. Accessory tissues of the male reproductive tract, in castrated rats, sensitively respond to chemicals with androgenic or antiandrogenic activities as an increase or decrease in absolute weights [14]. Pre-pubertal male rats are castrated before puberty and then allowed to recover for a period of 10 days. Animals are given the test compounds under evaluation as well as a known androgen (usually testosterone) as positive control for a period of 10 days. At the end, post-mortem androgen-sensitive tissues (ventral prostate, seminal vesicles, paired Cowper's glands, levator ani bulbocavernosus muscle and the glans penis) are obtained, weighed out and processed for histomorphometry if further analysis is needed. Details of the method can be found at an EPA guideline [201].

14 Test models for thyroid disrupting chemicals

It has been reported that *Xenopus laevis* serves as an ideal model organism to test thyroid axis disruption as thyroid hormones are highly conserved across vertebrates [202]. This is a 3-day exposure protocol in which evaluation of endocrine disruption of thyroid axis by the analysis of gene expression using wild-type *X. laevis* is described [202, 203].

There is also a cellular and molecular model to test thyroid disrupting chemicals *in vitro*. Thyroid signaling thorough Phosphatidylinositol 3 kinase (PI3K) has been reconstituted with recombinant receptors in mammalian cell lines [204]. Fluorescent reporters for PI3K activity can be used to qualitatively test large number of chemicals for disruption of PI3K stimulation by thyroid hormones [168].

15 Test models for Obesogens

A screening system for obesogenic compounds has been recently developed. This well-standardized adipogenesis model is based on 3 T3-L1 cell line [205]. Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor, acting as regulator for adipocyte differentiation and lipid metabolism [206]. PPARs can bind to unsaturated fatty acids and thus act as lipid sensors [116]. PPARγ agonists frequently induce adipogenesis. These nuclear transcriptional regulators can bind their response elements in the DNA and control gene expression. Details of the method has previously been described [205, 207].

16 Test models for adrenocortical disrupting chemicals

Cell lines have a prominent role in toxicity assessment, mechanistic investigations and high throughput analysis of chemicals for potential steroidogenesis disrupting activities. A comprehensive overview of cell lines available for studying effects of chemicals on adrenal and gonadal steroidogenesis and their use and limitations as test models has been reported [208].

H295R cell line derived from a human adenocarcinoma has been developed as an *in vitro* test model for evaluation of EDCs on adrenocortical function [156]. Protocols using H295R cell line to examine molecular mechanisms of steroidogenic pathway toxicity have been described [156, 209]. *In vivo* protocols using Zebrafish (*Danio rerio*) have recently been reported to test modifiers of adrenocortical function and examine effects of EDCs [210, 211].

Genotoxic and epigenetic analysis: Advanced molecular biology techniques such as single cell gel electrophoresis

assay (COMET), terminal transferase dUTP Nick End Labeling (TUNEL), sperm chromatin structure assay (SCSA) and in situ nick translation (ISNT) are employed to evaluate and monitor DNA damage in endocrine and gonadal cells [78]. COMET assay is a sensitive and practical tool to analyze DNA damage in various types of cells [36, 212, 213]. Methylation analysis by pyrosequencing is also employed to evaluate effects of EDCs on epigenetic changes in hormone sensitive and tumor related genes [214].

Today, sensitive, practical and cost-effective methods are needed to monitor and detect EDCs in human samples as well as in food and environmental samples. Detection of hundreds and thousands of chemicals in biological samples is time consuming, labor intensive and expensive. Therefore, we recommend use of screening methods (such as luciferase reporter gene assay) to determine biological actions (e.g. estrogenic or anti-estrogenic) of EDCs, and then, individual chemicals can be identified by chromatography and mass spectrometry in biological, food and environmental samples.

17 Human exposure to EDCs, risk assessment and strategy for prevention

EDCs are a global and ubiquitous problem [43]. It is difficult to assess the full impact of human exposure to EDCs because adverse effects develop latently and manifest at later ages, and in some people do not present. Most of the EDCs are lipophilic and accumulate in the adipose tissue, thus they have a very long half-life in the body. Latency between early exposures to EDCs and adult dysfunction/disease may be more than 50 years.

Main transmission route of EDCs occurs through ingestion of contaminated food. However, dermal contact with these environmental pollutants and inhalation of air-borne chemicals are two alternative ways of contamination to the human body. Therefore, exposure to EDCs occurs at home, in the office, on the farm, in the air we breathe, the food we ingest, and the water we consume. Biomonitoring studies show that almost all humans have a chemical body burden of these chemicals in their biological samples (adipose tissue, blood, urine, placenta and fetal blood). Response of individuals to chemical insult may be different. Some may experience overt toxicity, others may exhibit more subtle dysfunctions, and still others will not be affected at all [43]. These differences in responsiveness are due to the genetic background, environmental, occupational and life style factors, diseases, other stressors, eating habits and source of food consumed.

Identifying the risk associated with a single EDC is complex because humans are exposed to low doses of hundreds of chemicals starting at fetal life. These lifelong environmental exposures (known as the exposome) will need to be taken into consideration for comprehensive risk assessment studies. To investigate these complex interactions, methods based on omics technologies are being utilized [215].

For most EDCs, high doses are required for presentation of clear adverse effects. This causes controversy about the risks of life-long exposure to low doses of these pollutants. Sometimes very low doses of EDCs can exert potent effects on the endocrine and homeostatic systems. Why some EDCs produce non-traditional dose-response curves is not understood [216]. Human organisms are usually exposed to a mixture of chemicals, rather than a single contaminant. These features of exposure may generate a cocktail of endocrine disruptor effects with unknown consequences [158]. A recent experimental study showed that there was a different response in steroidogenesis and gene expression between the exposure to a mixture compared to the exposure to a single compound [217]. It is difficult to evaluate endocrine disruptor actions of a broad spectrum of chemicals in mixture exposures. Some EDCs have diverse hormonal activities, for example, the pesticide DDT is an ER agonist, whereas one of its metabolites exerts anti-androgenic effect [218]. Similarly, BPA is an estrogen agonist, but also antagonizes thyroid hormone receptor [219].

Timing of exposure to EDCs is evidently very important. Developing fetus and neonates are the most vulnerable to endocrine disruption [43], although EDCs may exert adverse effects on human health in other phases of the life cycle as well. Fetal development is a period of high plasticity which may be negatively influenced by exposure to environmental pollutants, resulting in disease later in life [32, 158]. Therefore, timing of exposure is of importance in assessment of the effects on the endocrine system.

EDCs are natural and synthetic substances with ubiquitous exposure in children and adults including pregnant women. Once these chemicals get into the food chain, it is difficult to get rid of them from the human body since most of the EDCs (particularly POPs) bioaccumulate in the fatty tissue. Plasticizers are not lipophilic and thus do not bioaccumulate in the body [66]. They are usually cleared from the organism in less than 24 h [55, 56]. However, we are continuously exposed to plasticizers such as BPA on a daily basis that causes a great deal of concern. Despite overwhelming evidence of its adverse effects on the endocrine system, there is still debate about safe exposure levels of BPA. Use of this plasticizer for production of baby bottles has been banned in Europe and USA since 2011 and 2012, respectively. Risk of exposure to POPs is well recognized, and some limitations are being implemented. For instance, maximum levels for dioxins and dioxin-like PCBs have been limited in milk and fat as 2.5 and 5.5 pg/g fat, respectively [220]. However, such a value has not been issued for estrogenic substances probably because their wide range of use and distribution. For pesticides, a maximum residue level (0.01 mg/kg) has been published that sets the amount of residues legally tolerated in food [221].

Table 1 A number of recommendations towards protection of individuals from EDCs and prevention strategies to be developed and implemented by policy makers / authorities are summarized

Recommendations for individuals for prevention of EDC exposure
• Pregnant women should especially avoid contact and inhalation of chemicals
• Children should be protected from chemical insult
• One should avoid consumption of chemical contaminated food and water
• Plastic, nonylphenols, petroleum products, and industrial fluids should not be burned
• Use of hot drinks such as tea and coffee in plastic cups should be avoided since plasticizers bleach into the fluid when warm.
• Warm milk should not be served in plastic bottles
• Use of glassware is recommended for consumption of warm food and drinks
• Warm pudding and similar food should not be served in plastic cups and plates
• Direct dermal contact to EDCs should be avoided
• Individuals should not swim in contaminated water
Recommendations for policy makers / authorities for prevention of EDC exposure
• Contamination of rivers, lakes and seas with chemicals should be prevented
• Drinking water should be monitored in terms of organochlorine chemicals
• Better water treatment technologies should be developed to detect and remove EDCs from drinking water
• Consumption of fish, meat, poultry products, milk and contaminated water should not be allowed
• Previously banned chemicals should be collected by trained personnel and stored safe and appropriately
• Burning of industrial solvents, plastics and chemicals should be prohibited
• Fire prevention should be provided especially at paint, textile and plastic manufacturing plants
• Any over production and use of plastic material should be limited, and regulations be developed
• Pools and drinking water reservoirs should not be over treated with chloride
• Fish, meat, dairy and poultry products should not be imported from countries/areas that are known to be chemically contaminated
• Chemically clean (organic) agriculture should be promoted
• Environmental pollution of EDCs should be prevented
• Remediation technologies for EDCs should be developed

A strategy for prevention of exposures to EDCs is urgently needed. This will require a political will to limit the use of these offending chemicals, and development and implementation of remediation technologies. Education programs in schools and hospitals (especially in endocrinology, pediatric and maternity clinics) will be helpful to improve general understanding of EDCs and the consequences of exposure to such pollutants, especially in early life. Clinical endocrinologists should be better educated about EDCs and their potential adverse effects on the human organism. Exposure to EDCs is more common among low-income individuals. Professional workers using pesticides, fungicides, paints and chemicals are at high risk of exposure to EDCs. Individuals at high risk should be informed and preventive measures be implemented to avoid extensive exposure. Informed individuals can act on a precautionary principle to reduce exposure of themselves and their children ahead of any regulatory actions, and thus have some degree of self-determination in their own exposure to EDCs. There are other preventive measures that are beyond individual capacity and therefore should be developed and implemented by policy makers and local governers. A number of recommendations towards protection of individuals and prevention strategies to be developed and implemented by policy makers / authorities are summarized in Table 1.

In this review, identification of EDCs, contamination routes, their adverse effects, underlying mechanisms and functional implications have been summarized. A translational approach is needed for the science of endocrine disruption. Biomonitoring and epidemiological studies should be carefully designed and conducted by collaborating basic and clinical scientists. It is also necessary to design basic research in *in vivo* and *in vitro* models and elucidate translational implications in humans. To avoid EDC exposure, prevention & protection strategies for individuals and policy makers / authorities should be developed and implemented.

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Compliance with ethical standards This is a review manuscript in which no human or animal studies were performed. All studies from our laboratories have been performed in accordance with international guidelines on animal welfare and human ethics.

Conflict of interest The authors declare that they have no conflict of interest.

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