Review on crosstalk and common mechanisms of endocrine disruptors: scaffolding to improve PBPK/PD model of EDCs mixture

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Abstract

Endocrine disruptor compounds (EDCs) are environment chemicals that cause harmful effect through multiple mechanisms, interfering with hormone system resulting in alteration of homeostasis, reproduction and developmental effect. Many of these EDCs have concurrent exposure with crosstalk and common mechanisms which may leads to dynamic interactions. To carry out risk assessment of EDCs' mixture, it is important to know the detailed toxic pathway, crosstalk of receptor and other factors like critical window of exposure. In this review, we summarise the major mechanism of actions of EDCs with the different/same target organs interfering with same/different class of hormone by altering their synthesis, metabolism, binding and cellular action. To show the impact of EDCs on life stage development, a case study on female fertility affecting germ cell is illustrated. Based on this summarised discussion, major groups of EDCs are classified based on their target organ, mode of action and potential risk. Finally, a conceptual model of pharmacodynamics interaction is proposed to integrate the crosstalk and common mechanism that modulates estrogen into the predictive mixture dosimetry model with dynamic interaction of mixture. This review will provide new insight for EDCs' risk assessment and can be used to develop next generation PBPK/PD models for EDCs' mixture analysis.

Keywords: Endocrine disruptor compounds (EDCs); toxicity mechanism; mixture interaction; common mechanism; crosstalk; PBPK/PD models.

List of Abbreviations

3MC: 3-methylcholanthrene

5α-R: 5- alpha reductase

ACTH: adrenocorticotropic hormone

Ahr: aryl hydrocarbon receptor

Ahrr: aryl hydrocarbon receptor repressor

AKT: serine/threonine kinase

AMH: anti-mullerian hormone

AMPO: ammonium Perflurooctane

ARC: arucate cell

Arnt: aryl nuclear translocator

AVPV: anteroventral periventricular nucleus

BAX: BCL2 associated protein

BCL2: apoptosis regulator

BMP: bone morphogenetic protein

BPA: bisphenol A

CAR: constitutive androstane receptor

CREB: cAMP response-element–binding protein

Cx43: connexin X 43

CYP1A1: cytochrome enzyme A

CYP1B1: cytochrome enzyme B

CYP19A: aromatase enzyme

CYP450scc: cytochrome p450 side chain cleavage

DBT: dibutylin

DEHP: diethylhexyl phthalate

DTCs: dithioarbamate chemicals

ERE: estrogen response element

E2: estrogen

FAK: focal adhesion kinase

Fas- membrane protein

FasL: fas ligand

Figla: factor in the germline alpha

FOXO3: forkhead box proteins

FSH: follicle stimulating hormone

GATA4: transcription factor

GDF: growth differentiation factor

GH: growth hormone

GJ: gap junction

GJA1: gap junction alpha protein

GnRH: gonadotropin releasing hormone

GVBD: germinal vesicle migration and breakdown

HAT: histone acetyl-transferase

HPA- hypothalamus pituitary adrenal axis

HDAC: histone deacetylases

HMT: histone methyl transferase

HPOA: hypothalamus preoptic nucleus

HSDs: hydroxysteroid dehydrogenases

HSP90: heat shock protein 90

IGF-1: insulin growth factor

IGFR: insulin growth factor recptor

Igf2r: insulin like growth factor 2

INH: inhibin

IP3-DAG: inositol triphosphate-diacyglycerol

LH: luteinizing hormone

LHR: luteinizing hormone receptor

LHX8: LIM homeobox 8

LXR: liver X receptor

LXR: liver X receptor

MAPK: mitogen activated protein kinase

MEHP: mono (2-ethylhexyl) phthalate

MMP2: metalloproteinase 2

NCoA: nuclear coactivator

NCoR: nuclear corepressor

NF-kB: nuclear factor k B

NOBOX: newborn ovary homeobox

NR: notch receptor

p160/SRC: steroid receptor coactivator

P23: protein 23

P4: progesterone

PR: progesterone receptor

PBPK/PD : Physiological based Pharmacokinetics/Pharmacodynamics modeling

PBR: peripheral type Benzodiazepine receptor

PCBs: polychlorinated biphenyl

PCDDs: polychlorinated dibenzodioxins

Peg3: paternal express gene 3

PEPCK: phosphoenolpyruvate carboxykinase

PFASs: poly-fluorinated alkyl substances

PI3: phosphatidylinositol 3-kinase

PMG: primordial germ cell

PPARs: peroxisome proliferator activated receptors

PTEN: phosphatase and tensin homolog

PXR: pregnane X receptor

RIP140: receptor interacting protein

ROS: reactive oxygen species

RXR: retinoid X receptor

SDM: sexual dimorphism

SF:1-steroidogenesis factor 1

SHBG: steroid hormone binding globulin

SMRT: silencing mediator for retinoid or thyroid-hormone receptors

Sohlh2: spermatogenesis and oogenesis helix-loop-helix 2

SREBP 2: sterol Response Element Binding Protein 2

SREBP1c : sterol Response Element Binding Protein 1c

StAR: steroid acute regulatory protein

SUG 1: suppressor for gal 1

SULTs: sulphotransferase enzyme

TAT: tyrosine aminotransferase

TBG: thyroid binding globulin

TBT: tributyltin

TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin

TCPOBOP : 1, 4-bis- [2-(3, 5,-dichloropyridyloxy)] benzene

TH: thyroid hormone

TJ: tight junction

TNF α : tumor necrosis factor α

TPT: triphenyltin

TRAIL: TNF:related apoptosis:inducing ligand

TRs: thyroid receptor

TSPO: translocator protein

UDPGT1A1: uridine diphosphate glucuronic transferase enzyme

VCL: vocal adhesion molecule vinculin

VEGF: vascular endothelial growth factor

VTG: vitellogenin

XAP2: X-associated protein 2

ZO-1: zonula occludens-1

1. Introduction

U.S. EPA define endocrine disruptor compounds (EDCs) as exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process (Kavlock et al., 1996). The WHO extended this definition linking EDCs to adverse health outcomes in an intact organism, or its progeny or subpopulation (WHO, 2002). The Endocrine Society describe EDCs as chemicals that interferes with any aspect of hormone action (Gore et al., 2014). EDCs can be found in daily uses products such as detergents, food cans, plastic bottles, children toys, flame retardants, cosmetics, and processed food (Clarkson, 1995; Rudel and Perovich, 2010). The EDCs interfere with hormone kinetics and its dynamic causing alteration in hormone level or expression of hormone responsive element (Crisp et al., 1998).

The aim of hormones is to execute its specific task on specific time with specific amount. There are many studies which link hormone alteration to different diseases outcomes. For example, low testosterone and SHBG level are the early biomarker for the risk of metabolic syndrome (Kupelian et al., 2006); alteration of E2, ERα , PR and the aromatase enzyme are strongly linked with endometriosis and infertility (Kitawaki et al., 2002); alteration in FSH, LH, inhibin B, and testosterone level is associated with decrease sperm quality (Meeker et al., 2006). Earlier assumption that EDCs and hormones would yield the same responses in different cell lines or tissues was found wrong. Now it is well known that EDCs have cell and tissues-specific responses (Lackey et al., 2001). Even at very low concentration, EDCs can produce significant endocrine disruptive action (Vom Saal and Hughs., 2005; Vandenberg et al., 2012) which challenges classical dose response curve at significantly high doses. Further, EDCs show disparate response at different life-stage dependent physiological concentrations of hormone, challenging current risk assessments methodologies which are not in consonance with life-stage changes (Welshons et al., 2003; Vandenberg et al., 2013). For instance, a study from Ohtake et al., (2003) showed that EDCs can produce contrary response based on physiological stage of prepubertal and pubertal. The interference of EDCs with developmental stages (prenatal-postnatal-early childhood-adulthood) and reproductive stages showed time of exposure as an important factor to determine its potency as well as developmental effect (Haimes, 2009; Gore et al., 2014). For example, there are strong relationship of EDCs exposure affecting HPG axis system and alteration in the age of female puberty showing developmental effect (Wang et al., 2005; Euling et al., 2008). The biological marker like enzyme expression and hormone level can help in assessing developmental risk by knowing detail mode of action of EDCs (Rockett et al., 2003).

Human are subject to continuous and simultaneous exposure to EDCs via its surrounding environment and bioaccumulation becomes inevitable in many cases, which might cause permanent damage following physiological adaptation failure (Vandenberg et al., 2013). Several studies showed that chemicals at individual level

have no observed effect level (NOEL), when exposed simultaneously as a mixture shows adverse effect disproving the concept of NOEL and taking more attention towards mixture studies (Rajapakse et al., 2002; Silva et al., 2002). The successive use of PBPK model in field of toxicology is commendable since it has great advantage of predicting internal tissue dose by integrating experimental data (both in vivo and in vitro) and extrapolation across species (Caldwell et al., 2012). However, level of biomarker of exposure (internal tissue dose) is, in many case, not sufficient to predict the toxicity of chemicals and additionally, when the effect of chemical mixture for certain response deemed to have toxicodynamic interaction. . Moreover, many biological response is the convergence of multiple signaling pathways, eventually become vulnerable to multiple targets of EDCs. Incorporation of the relationship between the exposure at the sites of action and the response generated can extend PBPK model to PBPK/PD (Nestorov, 2007). The objective of this review (summarized in Figure 1) is to understand the mechanism of actions of EDCs which includes interaction of chemicals with molecular receptor, enzymes, proteins, gene regulatory mechanism or epigenetic process thus affecting biological system, including window of exposure. Besides, this review also investigates the normal endogenous pathway of hormone sidewise to better understand the physiology dependent EDCs' action. The last part of the review includes an example showing common as well as cross talk mechanism of EDCs mixture affecting estrogen kinetics. Improved understanding of common as well as crosstalk mode of action and categorization of chemical based on similar adverse outcomes may provide better scaffolding for integration of pharmacokinetics and pharmacodynamics into predictive mixture toxicological model of EDCs.

2. Molecular mechanism of EDCs on the endocrine system

In general, individual EDCs can affect the endocrine system accounting their synthesis to metabolism; receptor mediated action, various signaling pathway and crosstalk signaling between receptors. In this section, a summarized review of EDCs' effects on major hormones namely thyroid and steroids (corticosteroid and gonadal) is provided.

2.1. EDCs affecting thyroid hormone

Thyroid hormones (THs) are one of the integral parts of hormone system required for normal brain and somatic development. It has been seen that EDCs can disrupts the function of thyroid system possibly through multiple mechanisms such as synthesis, transports, and the receptors like TR, Ahr, CAR, PPAR and RXR, mediated function for subsequent action and metabolism of hormone. Various chemicals affect homeostasis of hormone including Perchlorates, PCBs, PCDDs and PCDFs (Zoeller, 2010). Perchlorates inhibits uptake of iodide into thyroid follicle (Clewell et al., 2004). PCBs, PCDDs and PCDFs competitively bind with transthyretin impair transportation (Lans et al., 1994) and their affinity towards the Ahr receptor leads to increase metabolism of hormones (Poland and Knutson, 1982).

The toxicology pathway of EDCs via Ahr is shown in Figure 2; where Ahr receptor is present in the cytosol in conjugation with subunits like chaperon protein HSP90,

regulatory protein p23 and immunophilin like protein XAP2 (Perdew, 1988; Kazlauskas et al., 1999; Petrulis et al., 2000). Subsequently binding of EDCs with Ahr form complex followed by dissociation of Hsp90, P23 and XAP2 and translocation into the nucleus. In the nucleus, Ahr forms heterodimer complex with Arnt which then bind with XRE causing increase in expression of CYP1A1 and UDPGT1A; and finally leads to increase in metabolism of thyroid hormone (Hankinson, 1994; Birgelen et al., 1995). Simultaneously, there is feedback inhibition of Ahr transactivation by Ahrr (Mimura et al, 1999). Qatanani et al., (2005) reported that EDCs affinity towards CAR, can be other possible mechanism of metabolism of thyroid, that alters the UGTs and SULT mediated glucuronidation and sulfation of TH, respectively.

BPA has been reported as anti-thyroid agent that is mediated via multiple molecular mechanisms, mainly involved in altering receptor gene expression and dynamic stability. It decreases the TRα, TRβ mRNAs level and subsequently suppress RXR gene expression which is a heterodimer partner of TR. Additionally, it can also inhibits the binding of T3 to TR by recruiting N-CoR (Moriyama et al., 2002; Iwamuro et al., 2006). The isoform of TR remains in dynamically equilibrium state between inactive and active form to maintain the physiological action. The binding of EDCs with TR favors its inactive isoform (see Figure 3) via recruitment of (N-CoR). Subsequently, increase in HDAC, HMT, and HDM level induces the repression of target gene making TR inactive. In contrast, binding of thyroid to TR induces conformation changes and recruit coactivators of p160/SRC (steroid receptor coactivator). These coactivators have inherent histone acetylase activity that recruits complex like histone arginine methyltransferase (HMT), HAT and chromatin remodeling complex and form active homodimer or heterodimer complex with RXR (Ahuja et al., 2003; Yoon et al., 2005; Flamant et al., 2007). Juge-Aubry et al., (1995) mentioned that RXR was the common partner for both TRs and PPARs to form active heterodimers. Hence, the EDCs having affinity for PPARs or RXR could affect thyroid activity through crosstalk mechanism.

2.2. EDCs affecting steroid hormone

2.2.1. EDCs affecting corticosteroid hormone

Among corticosteroid hormones, glucocorticoids such as cortisol is produced in response to stress and is an integral part of HPA axis involved in cellular homeostasis and different metabolic processes. The enzymes that are responsible for the biosynthesis of these hormones mainly involved CYPs, HSDs and steroid reductases (Miller, 1988). The molecular mechanisms involved in biosynthesis are transfer of cholesterol to inner mitochondrial membrane by regulatory protein StAR (Manna and Stocco, 2005) and conversion of cholesterol to pregnenolone by CYP11A or CYP450scc (Parker and Schimmer, 1995; Manna and Stocco, 2005). Subsequent action of CYP17A and HSDs enzyme accomplish the glucocorticoid synthesis.

The interconversion of cortisol (active) to cortisone (inactive) involves two isoform of 11β-HSD namely 11β-HSD1 and 11β-HSD2 (Krozowski et al., 1999). This interconversion plays an important role in regulating central adiposity (Stewart et al., 1999) and protecting developing fetus from glucocorticoid excess (Krozowski et al.,

1995). The EDCs like PFASs, TBT, TPT and dithiocarbmates inhibit 11β-HSD2 isoform (Atanasov et al., 2003; Ohshima et al., 2005; Zhao et al., 2011) , and their exposure during pregnancy stage has been found to alter normal fetus development. Wang et al., (2012) mentioned the role of BPA on increased expression of 11β-HSD1, results in higher cortisol level, increase lipoprotein lipase and PPAR- γ which lead to increased adipocyte differentiation. The expression of PEPCK and TAT, well characterised metabolic response of glucocorticoid, was shown to be inhibited by DBT which decrease affinity of glucocorticoid towards its receptor (Gumy et al., 2008). Furthermore, one of the metabolic pathways of steroid involves PXR, a xenobiotic receptor which regulates CYP3A expression. Chemicals like phthalic acid and nonylphenol inhibit PXR degradation, thus enhancing CYP3A expression which leads to alteration in metabolism of steroid hormones (Masuyama et al., 2000, 2002).

2.2.2. EDCs affecting gonadal hormone

The effect of EDCs on human reproductive system has been linked with infertility, mediated through diverse mechanism that includes: altering gonadal steroidogenesis, affecting HPA axis and feed-back mechanism, altering receptor biology, crosstalk of receptor signaling, and direct organ toxicity. For the steroidogenesis, cholesterol is the main precursor which can be affected by the EDCs that alters receptor like PPARα and PXR which regulates transporter protein, such as Translocator protein (TSPO) or peripheral type Benzodiazepine receptor (PBR) that transport cholesterol from cytosol to the mitochondria (Hauet et al., 2005; Fan and Papadopoulos, 2012) and the metabolism of cholesterol by regulating transcription of rat CYP7A1 (cholesterol 7α-hydroxylase) gene (Marrapodi and Chiang, 2000; Staudinger et al., 2001; Li et al., 2011).

Moreover, the involvement of many supplementary pathways initiated via different receptor likes GHR, IGF-1 and (RXR/ TR) which regulate the function of steroidogenic enzyme and the affinity of EDCs towards these receptors, makes toxicity mechanism more complex (Chandrashekar and Bartke, 1993; Xu et al., 1995; Hull and Harvey, 2000; Manna et al., 2001; N'Diaye et al., 2002). In addition to that, the central system HPG axis which regulates gonadal cell plays an important role in normal reproductive development process. At the hypothalamic level, kisspeptin neurons express both, ligand KiSS-1 and its receptor GPR54 that regulates the release of GnRH in pituitary which in turn control expression of FSHR and LHR in gonadal cell. The kisspeptin neurons also express ER-α which involves in feedback inhibition of GnRH in response to estrogen stimulation. This feed forward mechanism holds important role during normal fertility cycle of pre-ovulatory to ovulatory phase (Roseweir and Millar, 2009; Silveira et al., 2010; Hameed et al., 2011). It has been shown in rodent models that exposure of BPA affects HPG axis with different mechanism depending on life stage of exposure; at prepubertal stage damages kisspeptin neuron and at puberty stage alters ERαmRNA expression (Ceccarelli et al., 2007; Patisaul et al., 2009). Xi et al., (2011) showed that the involvement of BPA on transcript levels of GnRH and FSH in the male and female pup via altering Kiss-1 mRNA expressions further supports the notion of multilevel mechanism of EDCs.

Boberg et al., (2008) reported that exposure to phthalates causes the reduction of anogenital distance, sign of male infertility, via the reduction of leptin level which supports the concept of leptin regulation of LH and FSH via leptin-kisspeptin-GnRH pathway (Neurons et al., 1999; Luque et al., 2007). The Leptin synthesis was also found to be inhibited by cadmium exposure (Stasenko et al., 2010). In addition to that the local gonadal enzyme CYP19A (aromatase) catalyses the androgen to estrogen conversion to balance androgen-estrogen level which is the prerequisite for the normal fertility in both male and female (Simpson et al. 1994). Several studies have been reported TBT inhibition of aromatase enzyme in granulosa cell results in imposex affecting fertility (Saitoh et al. 2001; Heidrich et al. 2001). Many studies have shown the EDCs dual action regards to estrogen level (Ohtake et al., 2003, 2007). For instance dioxins exposure at prepubertal stage, shows estrogenic activity via enhancing binding of ERα to ERE. However at pubertal stage, dioxin-receptor complex repressE2 bound ER function leading to antiestrogenic effects (Ohtake et al., 2003). In another study, Ohtake et al., (2007) reported the antiestrogenic activity of EDCs like TCDD and 3MC via activation of E3 ubiquitin ligase pathway that results in degradation of ERα and Ahr. In contrast to antiestrogenic activity, certain EDCs increase bioavailability of estrogens via inhibiting principle of estrogen sulphotransferase (SULT1E1) enzyme which causes inactivation of E2 (Kester et al., 2002).

The male sex hormone testosterone biosynthesis has been shown to be affected by TCDD and PFOA via different mechanism of action that involves altering signaling pathway, regulating expression of enzyme or direct inhibition of enzyme involved in steroidogenesis (Fukuzawa et al., 2004; Lai et al., 2005a; Shi et al., 2009; Zhao et al., 2010; Wan et al., 2011). Saunders et al., (1997) reported that exposure of pregnant mother to octyl phenol, decreases the level of testosterone in the fetal rat testis via altering the expression of CYP17α-hydroxylase/C17-20 lyase and steroidogenesis factor 1 (SF-1) leading to development reproductive disorder. The local hormone like AMH responsible for sexual differentiation in fetus during embryogenesis also nurture the testosterone by increasing prenatal proliferation of leydig cell and maintain the prepubertal stage in male. In parallel, developmental exposure of BPA and PCBs are linked to decrease level of AMH , LHR ,17β HSD3 and reduced aromatase activity in hypothalamus, affecting sexual maturation (Lee and Donahoe, 1993; Hany et al., 1999; Rey et al., 2003; Nanjappa et al., 2012). In addition to that, TBT or TPT are found to inhibit both 5α-R1 and 5α-R2 isozymes, responsible for production of active androgen (Svechnikov et al., 2010), affecting male sexual characterization (Doering et al. 2002). Castro et al., (2013) found similar results for BPA, reporting inhibition of both 5α reductases at their synthesis level. Simultaneous exposure of both chemicals (TBT and BPA) could lead to more impact on male fertility. Moreover, exposure to EDCs has shown to induce reproductive toxicity by damaging the integrity of blood testes barrier (BTB) in sertoli cell that causes impairment in spermatogenesis (Cheng et al., 2011).

The EDCs like BPA, PFOS, DEHP and cadmium induced reproductive toxicity is found to be mediated via altering MAPK, PI3K/c-Src/FAK, p38 MAPK and ROS signaling pathway leading to alteration in synthesis and metabolism of different protein likes

occludin, ZO-1, Cx43 and catenin affecting BTB integrity (Chitra et al., 2003; Sobarzo et al., 2006; Li et al., 2009; Siu et al., 2009; Cheng et al., 2011; Wong and Cheng, 2011; Qiu et al., 2013; Ansoumane et al., 2014). It has also been found that Sertoli cells have functional Ahr, responsible for TCDD dose-dependent toxicity that alters mRNA level of testin, aromatase, sertolin and MIS which are important for germ cell development (Lai etal. 2005). Phthalates are well characterized as reproductive toxic agents that causes apoptosis of germ cell by activating caspase pathway which includes: activation of fas by increased expression of fasl (Richburg and Boekelheide, 1996; Lee et al., 1999; Richburg et al., 1999; Koji et al., 2001), accumulation of lipid in somatic cells via increased LXRα mRNA expression (Muczynski et al., 2012) and downregulation of both GJA1 and vocal adhesion molecule vinculin (VCL) by increasing MMP2(Yao et al., 2012). Subsequently, activation of NFkB via increase expression of TRAIL-R1(DRP4) and TRAIL-R2 (DRP5) leads to increase apoptosis of germ cell without modification of their proliferation (Giammona 2002; Lambrot et al. 2009). Figure 4 shows the mechanism of phthalates causing germ cell apoptosis in fetus.

3. Effect of EDCs in different window of exposure: case study on female fertility

It has been shown that EDCs have disparate response at different life-stage, depending on the physiological concentrations of hormone (Ohtake et al., 2003). However, primary concerns for female fertility are exposure to EDCs at prenatal and postnatal stages, which are at higher risk of reproductive failure as well as metabolic disorder and hormonal disorders in their later life. EDCs can alter normal cellular and tissue development and function through their interference in developmental programming of body (Schug et al., 2011). To study the life stage risk assessment on fertility, it is very important to know the detailed mechanism behind development of germ cell into mature oocyte. This involves complex and sequential biological network of signaling pathway.

3.1. Physiology of development of germ cell into mature oocyte

During epigenetic reprogramming of germ cell, at the very first step, involves DNA demethylation to regain differentiation totipotency which subsequently undergoes mitotic division without completing cytokinesis to the formation of germ cell cyst (Pepling and Spradling, 1998). Before birth, germ cells go through meiosis and arrest in diplotene phase of meiotic prophase until puberty comes. Meanwhile germ cell cyst undergoes apoptosis followed by surrounding of pregranulosa cell forming primordial follicles (Borum, 1961; Pepling and Spradling, 2001). After forming primordial follicles, estrogens play a role in maintaining these follicles pool by inhibiting oocyte nest breakdown through inhibition of BCL-2 gene transcription via both genomic and nongenomic pathway (Perillo et al., 2000; Chen et al., 2007, 2009).

Moreover, additional pathways are also involved for the regulation of primordial follicles which involves Notch signaling, and KIT-KL pathway. Notch signaling activation involves expression of Jagged1 and Jagged2 (ligand), in germ cells and Notch2 (ligand), in granulosa cells to form a receptor ligand complex. The proteolytic

cleavage of this complex by γ-secretase produces intracellular domain of Notch (NICD) which translocate into the nucleus and interacts with the CSL family to form the complex. This complex recruits histone acetylase and regulates the expression of LHX8, NOBOX, Figla and Sohlh2 involved in formation of primordial follicles (Baron, 2003; Shih and Wang, 2007; Chen et al., 2014; Vanorny et al., 2014). KIT receptor expressed in oocyte and the KIT ligand is present in both oocyte and primordial follicle, help in initiation and progression of follicular development (Parrott and Skinner, 1999) via the activation of the MAPK pathway (Jones and Pepling, 2013). GDF9 increases kit ligand mRNA expression and thus promotes the progression of primary follicle development (Nilsson and Skinner, 2002). BMP4 and BMP7 play a major role in survival and growth of primordial follicle to primary follicle by decreasing KL and TGF-α expression respectively (Nilsson and Skinner, 2003; Lee et al., 2004). Cx43 expressed in both cumulus and granulosa cell play an important role in paracrine signalling and gap junctional intercellular communication between cumulus cell and follicular cell providing follicular development and oocyte quality (Ackert et al., 2001; Gittens et al., 2005; Wang et al., 2009). BMP4, BMP7 and BMP15 downregulate Cx43 in human granulosa cell via smad pathway and thus decreases the gap junctional intercellular communication leading to prevention of premature luteinization (Chang et al., 2013; H. M. Chang et al., 2014).

The interplay between paracrine hormones is very important for the transition of primordial follicle to primary follicle to become a mature oocyte. AMH inhibits primordial follicles to enter the pool of growing follicles (Durlinger et al., 1999) by decreasing expression of inhibin (Themmen and Themmen, 2009). Billiar et al., (2003) also reported the inhibition of expression of inhibin by the estrogen in pregranulosa and oocyte. Thus, estrogens play an important role in regulating inhibin and follicular development. The TGF-β signaling involves GATA-4 and Smad-3 coordination for activation the inhibin (Anttonen et al., 2006). Androgens play an important role in follicle development via increasing expression of, FOXO-3, GDF9 through PI3/AKT pathway, and, KIT/KL through genomic pathway during primordial follicle to primary follicle stage. Specifically, during development of primary follicle to antral stage, it inhibits proapoptotic proteins and stimulates FSH mRNA expression, cAMP and p450scc through both genomic and non genomic i.e. MAPK/ERK pathway which in turn stimulates aromatase enzyme (Prizant et al., 2014). FSH stimulates LHR expression, (Richards et al., 1976) inhibin B production (Lee et al., 1982), and induces aromatase activity in the granulosa cells, results in more estradiol level (Short, 1962; Richards et al., 1976; Hillier et al., 1981). Moreover, most FSH sensitive called dominant follicle produces the highest levels of inhibin B and estradiol which in turn causes feedback inhibition of FSH production, required for growth of remnant follicles (Hirshfield and Midgley, 1978). After selection of dominant follicle, subsequently progesterone causes germinal vesicle migration and breakdown (GVBD) for resumption of meiosis at puberty by activating p53 and E2F transcription factor 1 (Garcia-reyero et al., 2015) leading to ovulation. The fertilization of ovum results in formation of zygote and matured follicle after releasing ovum called lutein cell which secretes VEGF. It

prolongs the lutein cell function that maintains the progesterone level important for pregnancy development. VEGF function is regulated via PPARγ (Fraser et al., 2000; Kaczmarek et al., 2005).

3.2. EDCs interaction with target molecules and its pathway

Exposure to Lindane, PCBs and PAHs to embryo has been linked with premature reproductive ageing by causing the apoptosis of germ cell through different pathways such as activation of caspase-3 and poly-ADP ribose polymerase cleavage (PPAR) by Lindane and activation of BAX via Ahr by PAHs (Ronnback and de Rooij, 1994; Matikainen et al., 2002; La Sala et al., 2009; Kee et al., 2010). Phthalates exposure induce primordial follicle recruitment via activation of PI3K/AKT pathway, resulting in premature ovarian follicle and infertility (Hannon et al., 2014). Moreover Castrillon et al., (2003) study supported that FOXO3A knock out mouse, leading to premature oocyte follicle which is regulated by the PTEN/PI3K/AKT pathway. Both, Phthalates and BPA lowers the expression of LHX8, Nobox, Figla, and Sohlh2, involved in oocyte survival and follicular recruitment to form primordial follicle. In addition to this both compounds alter epigenetic reprogramming of Lhx8 by preventing DNA demethylation (Zhang et al., 2012, 2014). However, BPA shows multiple mechanism of action, altering steroidogenesis and proliferation of granulosa cell such as: induction of PPARγ causing downregulation of FSH-stimulated IGF-1, SF-1, GATA4, aromatase, and E2 (Kwintkiewicz et al., 2010), decreases both StAR and P450scc mRNA impairing hormone production in the antral follicles (Peretz et al., 2011), activates nongenomic pathway of estrogen via PKA and PKG pathway associated with phosphorylation of transcription factor CREB and the cell cycle regulator Rb (Bouskine et al., 2009). Additionally, BPA delayed maturation of oocyte by inhibiting resumption of meiosis via altering ER expression, following hypomethylation of imprinted gene Igf2r, Peg3, and GVBD, (Chao et al., 2012). On the other hand, other EDCs like Methoxychlor inhibits follicular development by stimulating AMH (Uzumcu et al., 2006). This is further supported by the study of impairmaint of follicular development in neonates on exposure of estradiol benzoate found to be via increased expression of AMH (Ikeda et al., 2002). Moreover, Nagel et al., (1999) shown that BPA even at very low dose can affect sexual dimorphism of infants via its estrogenic action in brain. whereas in normal, prenatal estrogen form complex with Alpha fetoprotein, protecting the female brain from defeminization and masculinization (Bakker et al., 2006).

EDCs contamination in the human follicular micro-environment is associated with a lower chance of an oocyte to develop into a top-quality embryo, leading to lowering in fertilization rate (Petro et al., 2012). For instance, PCBs exposure affects oocyte quality and competence via multiple mechanisms; alters microtubule organization, mRNA polyadenylation levels, redistribution of cortical granules, mitochondrial disorganization, leading to polyspermy and affecting in transcript stability. It can also directly cause cumulus cell apoptosis which is communicator cell between oocyte and follicle mediated via Ahr signaling (Gandolfi et al., 2002; Brevini et al., 2005; Pocar et al., 2006). MEHP an endocrine disruptor inhibit embryonic genome activation (EGA)

initiation and maternal-effect genes resulting in the suppression of maternal-to-embryonic transition by generating ROS (Chu et al., 2013).

Figure 5 summarizes the life stage development of germ cell to oocyte and the possible targets of EDCs. In his turn, Figure 6 explains the complex signaling pathway for life stage development of germ cell maturation to oocyte.

4. Grouping strategy and conceptual model of PBPK/PD in assessing risk for chemical mixture

4.1. Grouping strategy

There are numerous classification of EDCs reported in the literature based on different criteria like pathway of exposure, level of exposure, target hormones, adverse effects, and diseases outcomes (Caserta et al., 2008; Wuttke et al., 2010; Craig et al., 2011; Schug et al., 2011; Casals-Casas and Desvergne, 2011; Vandenberg et al., 2012; Hampl et al., 2014). Ongoing discussion of the risk assessment for chemical mixture (EFSA, 2013) needs new grouping strategy which clusters EDCs based on their similar adverse outcomes via independent, cross talk and common interaction mechanism involving multiple organs and hormones. Similar prerequisite for cumulative risk assessment of chemical mixtures has been cited by EFSA (Kortenkamp, 2007; EFSA, 2013). This type of grouping strategy (based on similar adverse outcomes) could also help in making decision on whether to go for dose addition or response addition method for mixture interaction study (Culleres et al., 2008). A detail discussion on classification is beyond the scope of this review. However, a detail classification for selected chemicals is provided in Annex Table 1. Classification of EDCs proposed in this review is based on target organs, hormones, biomolecule (MOA) and adverse outcomes, which can provides basis for grouping strategy for mixture modelling. Proposed grouping strategy has been illustrated in Figure 7 by giving a small example of four chemicals (BPA, TCDD, Phthalates and PFOS). Some of these chemicals are categorized in one group for mixture study based on their similar adverse outcome including targets organs like thyroid gland and sertoli cell, and in another group with dissimilar mode of action (crosstalk) producing common adverse effect of altering thyroid action and decreasing sperm count, respectively. Similar grouping strategy has been followed in Figure.9 for the chemicals affecting female fertility.

4.2. Conceptual model of PBPK/PD

A chemical can alter hormone actions by targeting at the level of epigenetics-gene-enzyme/receptor followed by endogenous intracellular signaling pathway (Grün and Blumberg, 2006; Cruz et al., 2014). Therefore, the mixture of chemicals producing similar adverse outcomes via entirely different mode of action can be categorized in one group in order to analyze the combination effect. Furthermore, timing and level of exposure is also an important parameter which can make adverse effect temporary or permanent and has to be included when assessing risk (Fenton, 2006; Buck Louis et al., 2008; Palanza et al., 2016). Based on methodologies (Figure 7), we proposed a

conceptual model which brings the fate and the consequence of chemical mixture in the integrated risk assessment framework of exposome-internal exposure-biological effect to the adverse outcome (Figure 8).

At the dynamic level, integration of individual mechanisms to the dynamic interactions of mixture for assessing risk is still debatable (Lambert and Lipscomb, 2007; EFSA, 2013; Karri et al., 2016). Figure 9 shows a small example of hypothetical schematic model that integrates individual mode of actions based on their target molecule in a system based approach. It includes common, crosstalk as well as dissimilar mode of action based on their targets of common outcome. For instance, the dioxin-like chemicals, DBP, BPA, TOP and PAH-OH alter the estrogen action at different levels of peripheral as well as central mechanism. Their major targets include kisspeptin neuron, CYP19A (aromatase), SHBG, ER, Ahr, ERE CYPA1 and CYPB1 affecting estrogen and progesterone feed forward mechanism, consequently leading to risk of infertility. In fact, EDCs like DBP, BPA and TOP show similar mode of action via targeting CYP19A and SHBG. Dioxin-like substances exhibit dual role such as “antiestrogenic” via Ahr dependent CYPB1 mechanism and “estrogenic” via estrogen receptor showing crosstalk between ER and Ahr. PAH-OH and BPA can interact with other dioxin-like substances in respect to their targets via crosstalk between Ahr and SULTE1 altering metabolism of estrogen. BPA, PAH-OH and other dioxin-like substance are able to simultaneously interfere with endocrine system through multiple mechanisms. The mixture effects of these chemicals in system based model can be possible by considering estrogen, progesterone and ERE, as end point biomarker of infertility, and integrating available individual toxicological profile data into a dynamic mixture model of EDCs (PBPK/PD).

5. Summary & future perspective

We have summarized the effects of endocrine disruptors on thyroid, adrenal, and sex hormones accounting their effects on synthesis, metabolisms and actions. Mixture of chemicals can simultaneously interfere with multiple endocrine pathways via multiple mechanisms making mixture effect more pronounced than individual. The EDCs acting on certain hormone via multiple mechanisms (central or peripheral) can be grouped for risk assessment of mixture of chemicals, according with their similar adverse outcomes.

Most of the EDCs have non monotonic dose-response curve which is the major drawback when establishing a relationship between the exposure kinetics and elicited response (Vandenberg et al., 2012; Beausoleil et al., 2013; Yang et al., 2016). Additional challenges like multiple mechanisms, delayed response (time lag between exposure to adverse outcomes), dynamic interaction involving crosstalk and common mechanism, and transgenerational effect added more complexity in the quantitative risk assessment (Maffini et al., 2006; Matthiessen and Johnson, 2007; Rubin, 2011; Fowler et al., 2012).

However, understanding molecular mechanism of interaction of chemicals with endogenous molecule or pathway can explain the variability among chemicals for the

same adverse effect (Filby et al., 2007). For instance, BPA shows complex dose-response curve in concentration dependent model which could be explained by the fact that it alters the gene expression through genomic as well as nongenomic pathway (Takayanagi et al., 2006; Vandenberg et al., 2009; Vandenberg, 2014). Similarly, dioxin-like substance shows dual response that can be explained by availability of endogenous hormone and their action. The potential dynamic interaction may leads to change in the response curve in case of mixture of chemical, which can be explained by understanding different type of mechanistic interactions like crosstalk or similar or dissimilar MOAs as it has been explained in this review. Similarly, understanding latency of exposure (i.e. lag time between exposure and response) is important as in case of infertility disorder, which can only be detected after a certain age though exposure occurs at early stage of life.

Lots of experiments have been done on individual EDCs but it is very hard to find mixture level studies. Selecting chemicals and then optimizing dose for selected mixture for carrying animal experiment is another difficult task. To know the potency of individual chemical in mixture due to their complex interaction behavior at different levels, require large combinatorial experimental design. Normally this kind of experiments requires large number of animals which will be against the current ethical guideline of risk assessment (EU, 2010). However, tremendous development in in-vitro, in-silico techniques and emerging area like omics, generating lots of toxicological data leads to new era of quantitative risk assessment (Knudsen et al., 2015).

Incorporation of individual mechanism of chemicals into mixture model provides platform for assessment of combined risk produced by mixture of chemicals. Understanding individual mechanism and implementing those mechanisms in system based approach will help us in the development of mixture model. This will provide better understanding of the risk produced by chemical mixture exposure and it will further assist in designing animal experiment and optimization of dose which will reduce the use of animals. European Union, (2011) suggested concentration addition method for cumulative risk assessment of chemicals with similar or dissimilar mechanism of action by considering their common adverse outcomes. But response addition method for a common adverse effect is still not recommended.

Categorization of chemicals in same group according to similar adverse outcomes, accounting both similar as well as dissimilar mechanism (crosstalk) of action may provide sound basis for studying mixture toxicology. Based on this grouping strategy, addressing both kinetic and dynamic interaction of mixture and establishing a relationship between pharmacokinetic - pharmacodynamic- altered molecular events will give a better model to correlate the environment exposure with adverse outcomes. Finally, integrating individual mode of action of each chemical by the help of mathematic equation into advanced tools such as PBPK/PD would enable the simultaneous assessment of EDCs mixtures correlating concentration in various biological matrixes (blood, tissue, urine) with various end points (endocrine diseases). It will also help in finding the toxic equivalent dose of chemical eliciting similar adverse

effect. Similarly, timing and duration of exposure is another important factor which needs to be considered while assessing the risk. Integrating physiology of human body at different life stages and respective mode of action of EDCs will help in building life stage dynamic model. For example, dividing life stage into prenatal-postnatal-puberty-menopause and incorporating susceptible gene or receptor or protein at different life stage targeted by EDCs and physiological data provides a model able to predict the risk of infertility in females by exposure to these chemicals in different stage of life.

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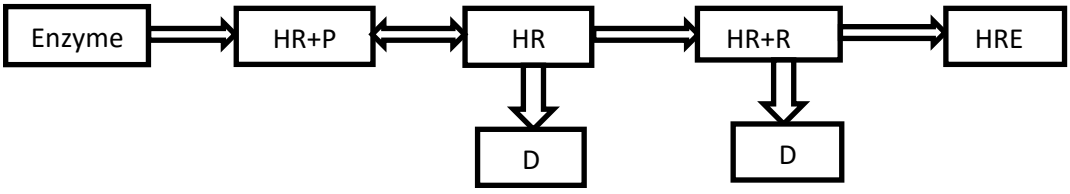


Figure 1: Effects of EDCs on hormone action at different level.

Enzyme responsible for hormone synthesis, HR- hormone, P- hormone binding protein, R- receptor, D- degradation of hormone and its receptor, HRE- hormone response element.

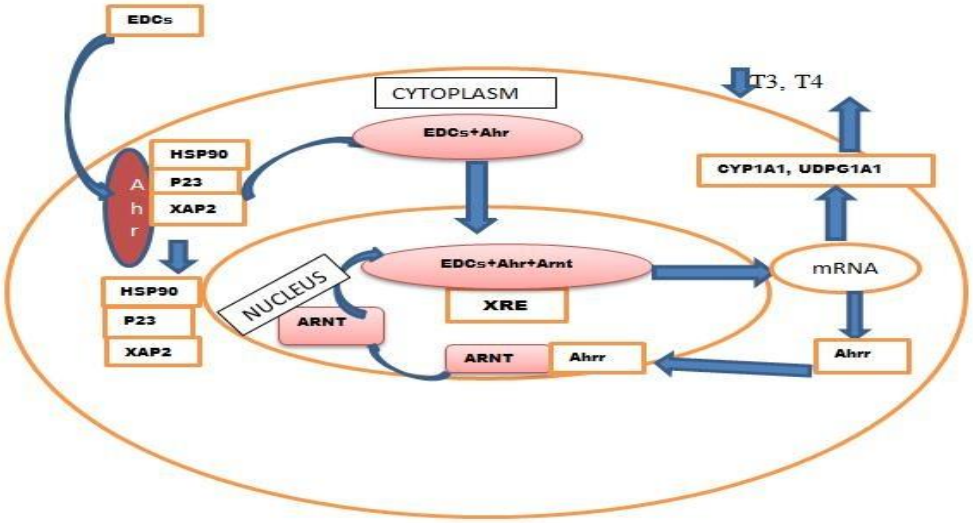


Figure 2: Summary of molecular mechanism of EDCs binding with Ahr-. The binding of EDCs with Ahr leads to translocation of Ahr receptor to the nucleus from cytoplasm following dissociation of chaperons, forming Ahr-Arnt complex. This complex binds with XRE (xenobiotic response element) causing induction of CYPs enzyme, enhancing metabolism of endogenous hormone.

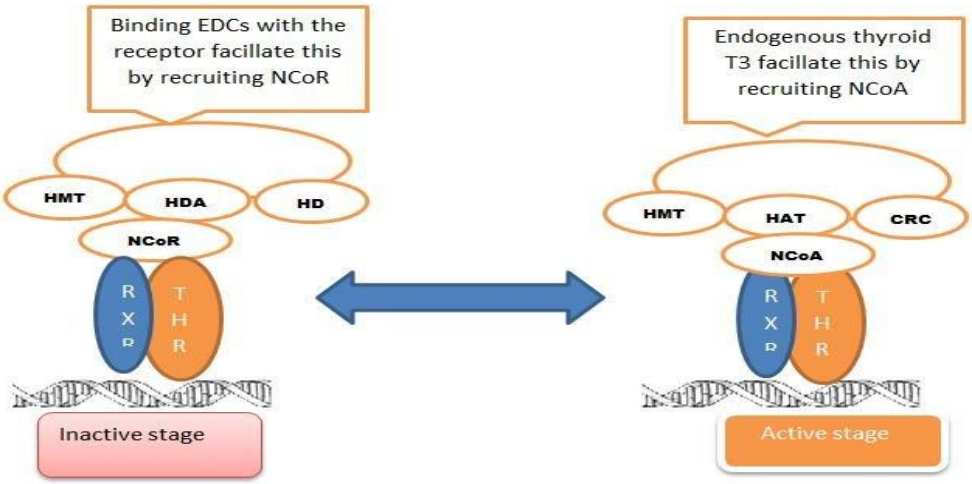


Figure 3: EDCs affecting dynamic state of receptor. Unliganded thyroid receptor resides in nucleus in inactive state by recruiting NCoR and thyroid binding facillates active stage by recruiting NCoA. Binding of EDCs with thyroid receptor induced conformational changes by recruiting NCoR facilitating its inactive stage.

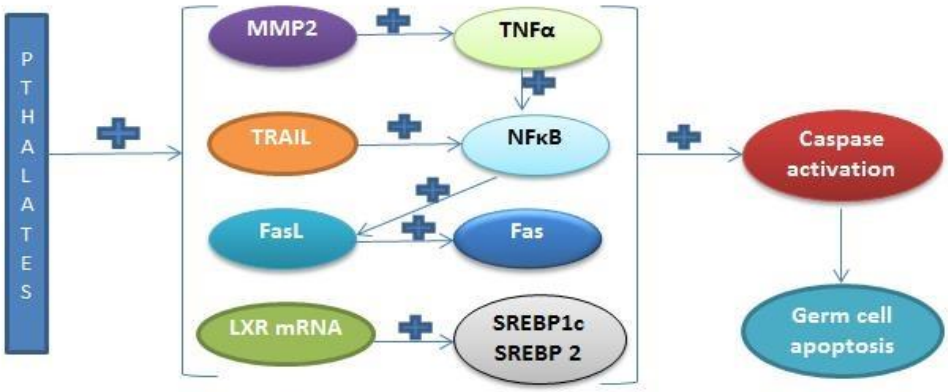


Figure 4: Mechanism of phthalates causing germ cell apoptosis in fetus. Phthalates exposure at tissue level causes activation of caspase pathway which lead to apoptosis of germ cell through interaction and activation of receptor and gene at cellular level.

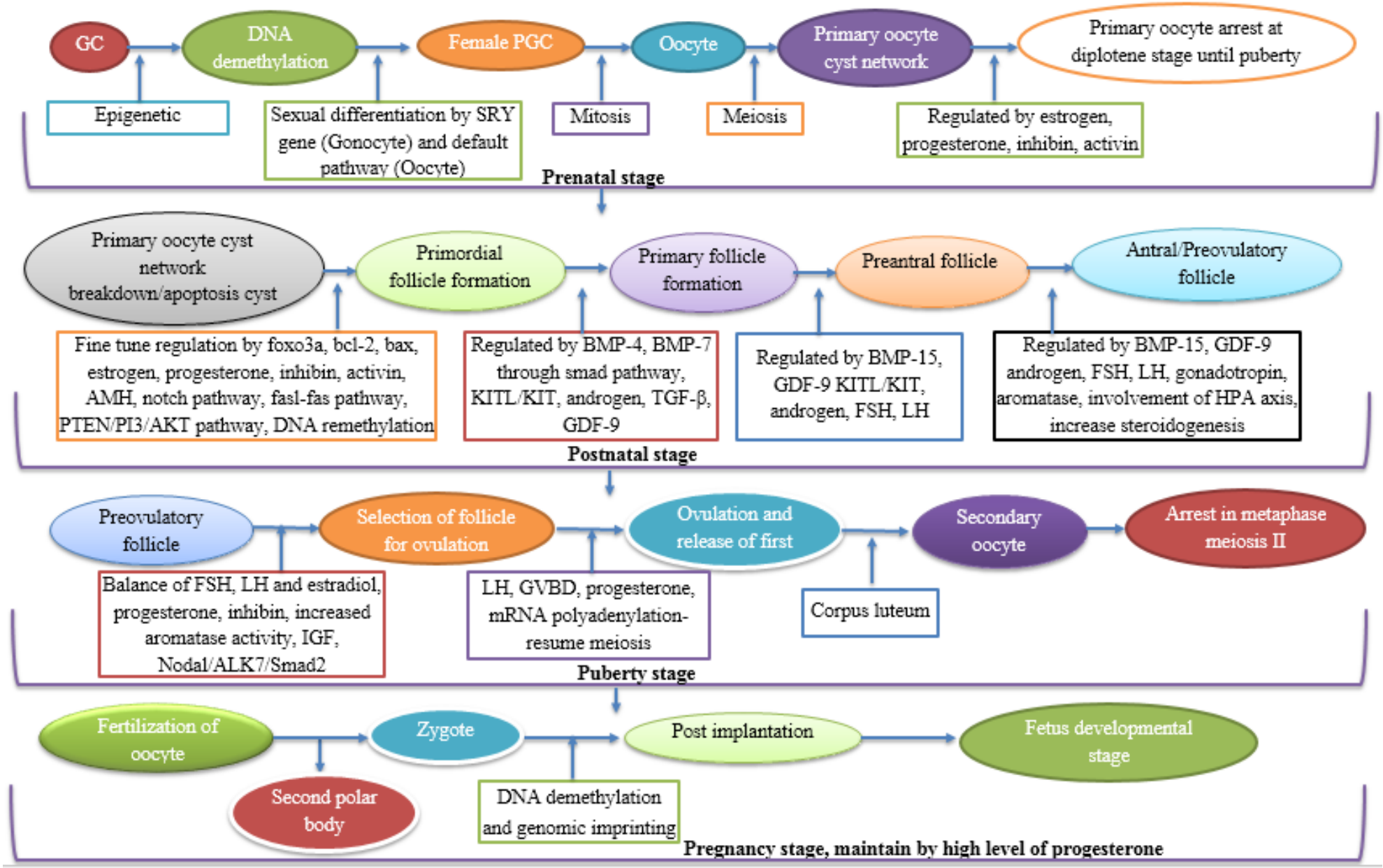

GC
DNA demethylation
Female PGC
Oocyte
Primary oocyte cyst network
Primary oocyte arrest at diplotene stage until puberty
Epigenetic
Sexual differentiation by SRY gene (Gonocyte) and default pathway (Oocyte)
Mitosis
Meiosis
Regulated by estrogen, progesterone, inhibin, activin
Prenatal stage
Primary oocyte cyst network breakdown/apoptosis cyst
Primordial follicle formation
Primary follicle formation
Preantral follicle
Antral/Preovulatory follicle
Fine tune regulation by foxo3a, bcl-2, bax, estrogen, progesterone, inhibin, activin, AMH, notch pathway, fasl-fas pathway, PTEN/PI3/AKT pathway, DNA remethylation
Regulated by BMP-4, BMP-7 through smad pathway, KITL/KIT, androgen, TGF-β, GDF-9
Regulated by BMP-15, GDF-9 KITL/KIT, androgen, FSH, LH
Regulated by BMP-15, GDF-9 androgen, FSH, LH, gonadotropin, aromatase, involvement of HPA axis, increase steroidogenesis
Postnatal stage
Preovulatory follicle
Selection of follicle for ovulation
Ovulation and release of first
Secondary oocyte
Arrest in metaphase meiosis II
Balance of FSH, LH and estradiol, progesterone, inhibin, increased aromatase activity, IGF, Nodal/ALK7/Smad2
LH, GVBD, progesterone, mRNA polyadenylation-resume meiosis
Corpus luteum
Puberty stage
Fertilization of oocyte
Zygote
Post implantation
Fetus developmental stage
Second polar body
DNA demethylation and genomic imprinting
Pregnancy stage, maintain by high level of progesterone

Figure 5: Life stage development of germ cell and the possible targets of EDCs. The germ cell, basis of future sexual life or transgenerational development, development of oocyte from germ cell starts at embryo stage. Exposure of EDCs to pregnant mother (F0) may cross placental barrier and affect embryonic germ cell in fetus (F1). This could lead to alteration in oocyte quality required for fertilization and transgenerational fetus development (F2). Every stage of development of germ cell to high quality oocyte, demands fine tune balance of endogenous level and interaction pathway. Categorizing development of germ in stages provides information on susceptible targets of EDCs during the journey of germ cell of fetus (F1) residing in mother embryo (F0) to high quality of oocyte, for development of transgenerational fetus (F2).

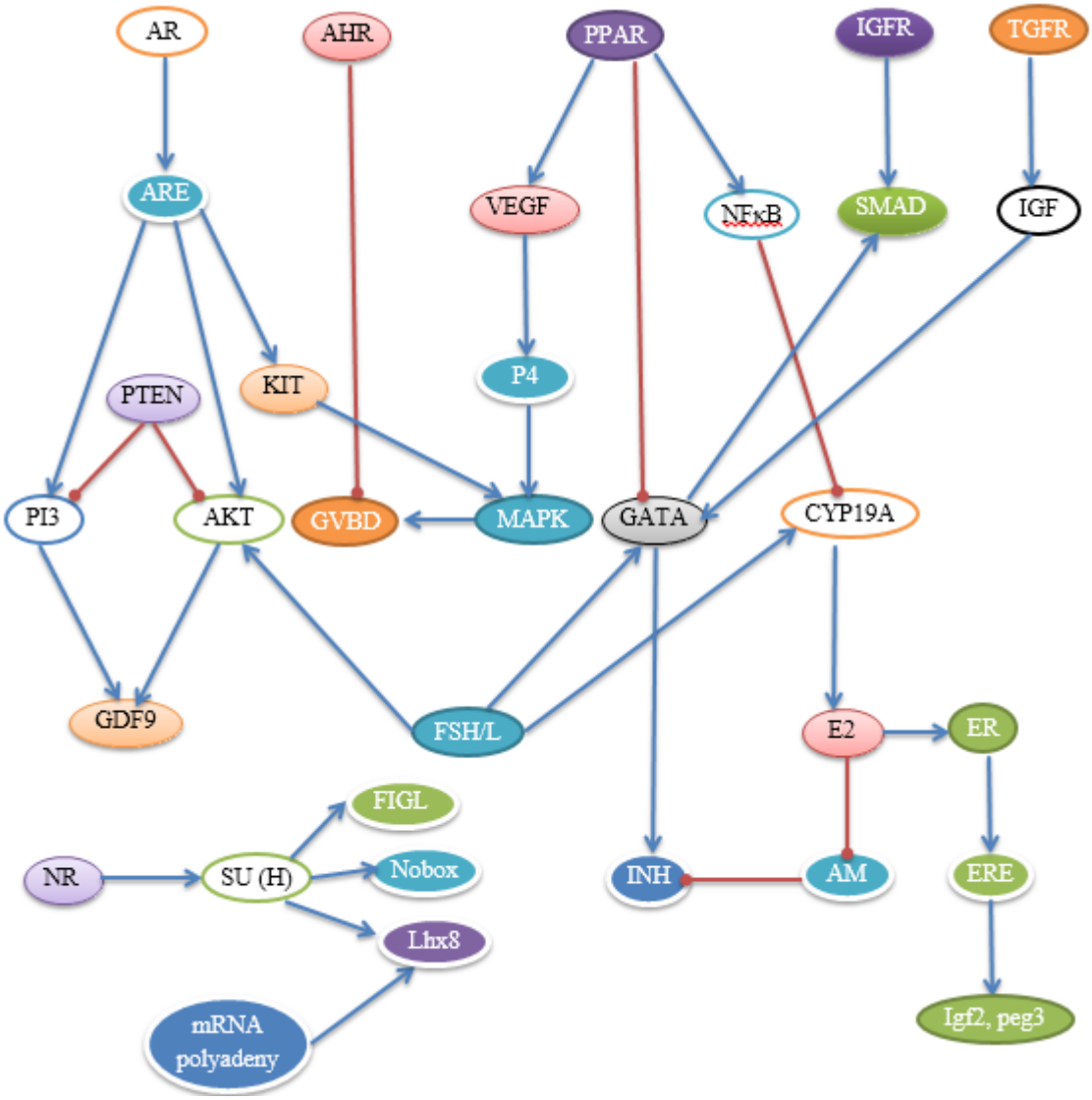
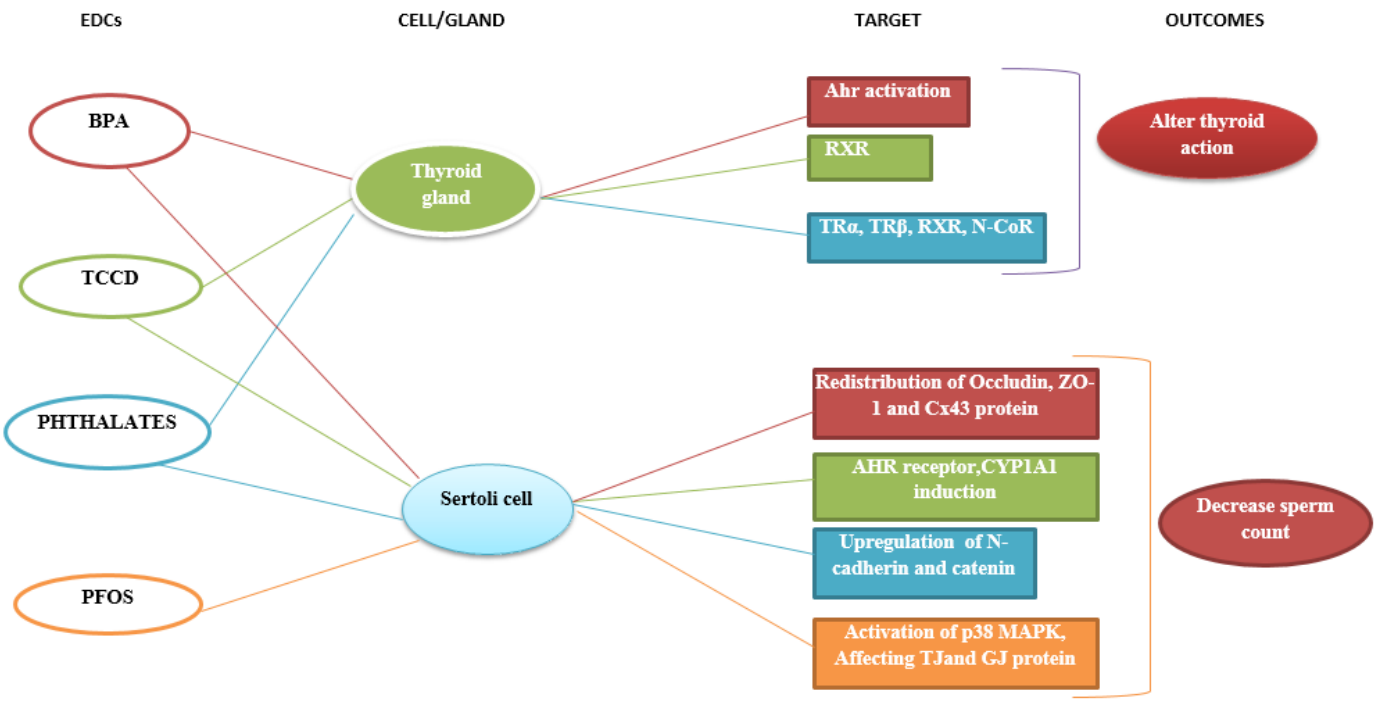


Figure 6: Signaling pathway for life stage development of germ cell to zygote. The figure depicts the different signaling pathways initiation via binding of endogenous molecule with receptors, which leads to inhibitory and stimulatory effect on signaling molecule following physiological demand for the development of germ cell into mature oocyte.



BPA- ——— , TCDD- ——— , Phthalates- ——— , PFOS- ———

Figure 7: Endocrine disruptor's classification on the basis of mode of action for selected chemicals (BPA, TCDD, Phthalates and PFOS), with different targets on thyroid and sertoli cell with common adverse effect in respective cell.

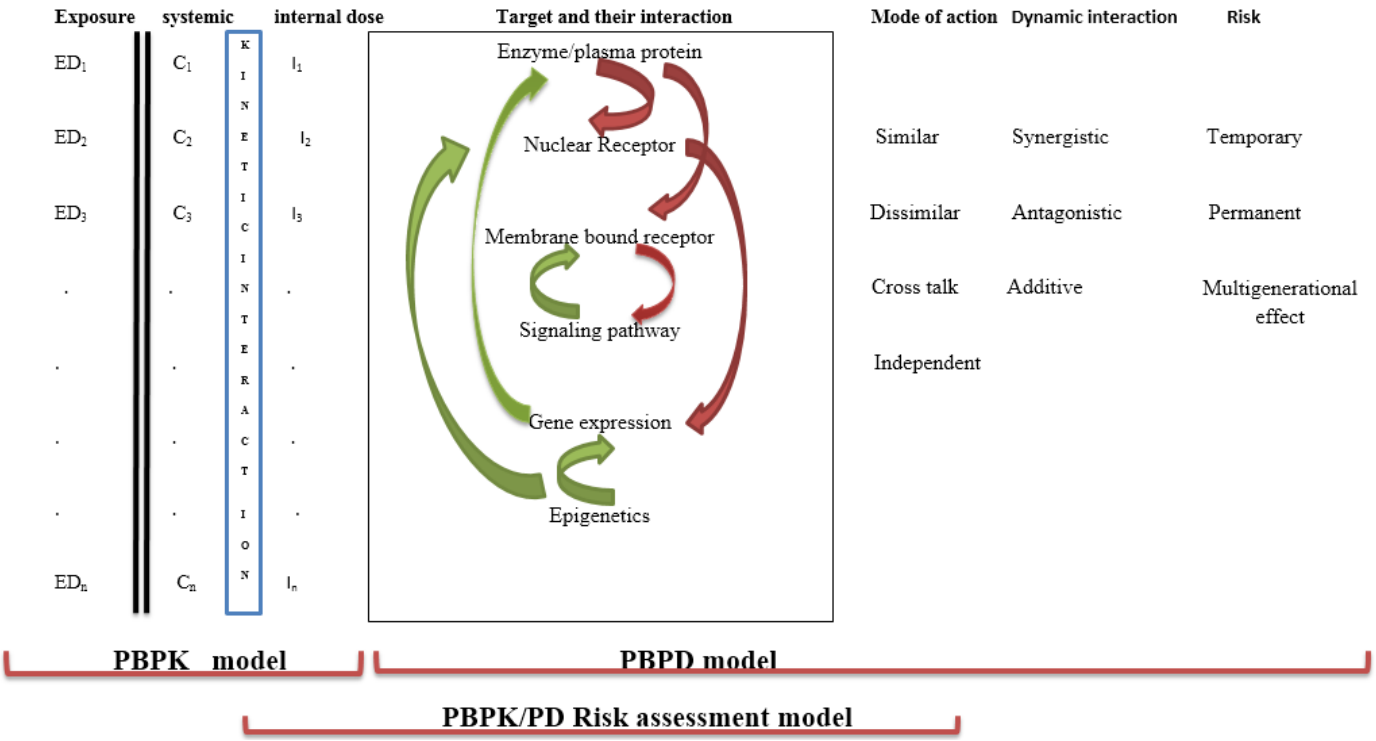


Figure 8: Conceptual model of PBPK/PD in assessing risk for chemical mixture (ED- endocrine disruptor exposure, C- concentration of ED in systemic circulation, I- concentration of ED in target organ or tissue, DI- dynamic interaction)

PBPK usually well describes time course of tissue level exposure of chemical relating environmental exposure by including their absorption, distribution, metabolism and excretion. At cellular level, the interaction of chemicals with endogenous biomolecules and their pathways which are interrelated with each other results in initiation of an event that could lead to adverse outcomes which can be describe by PBPD model. The integrated PBPK/PD can describe the kinetic as well as dynamic interaction of EDCs giving time course effect of chemicals.

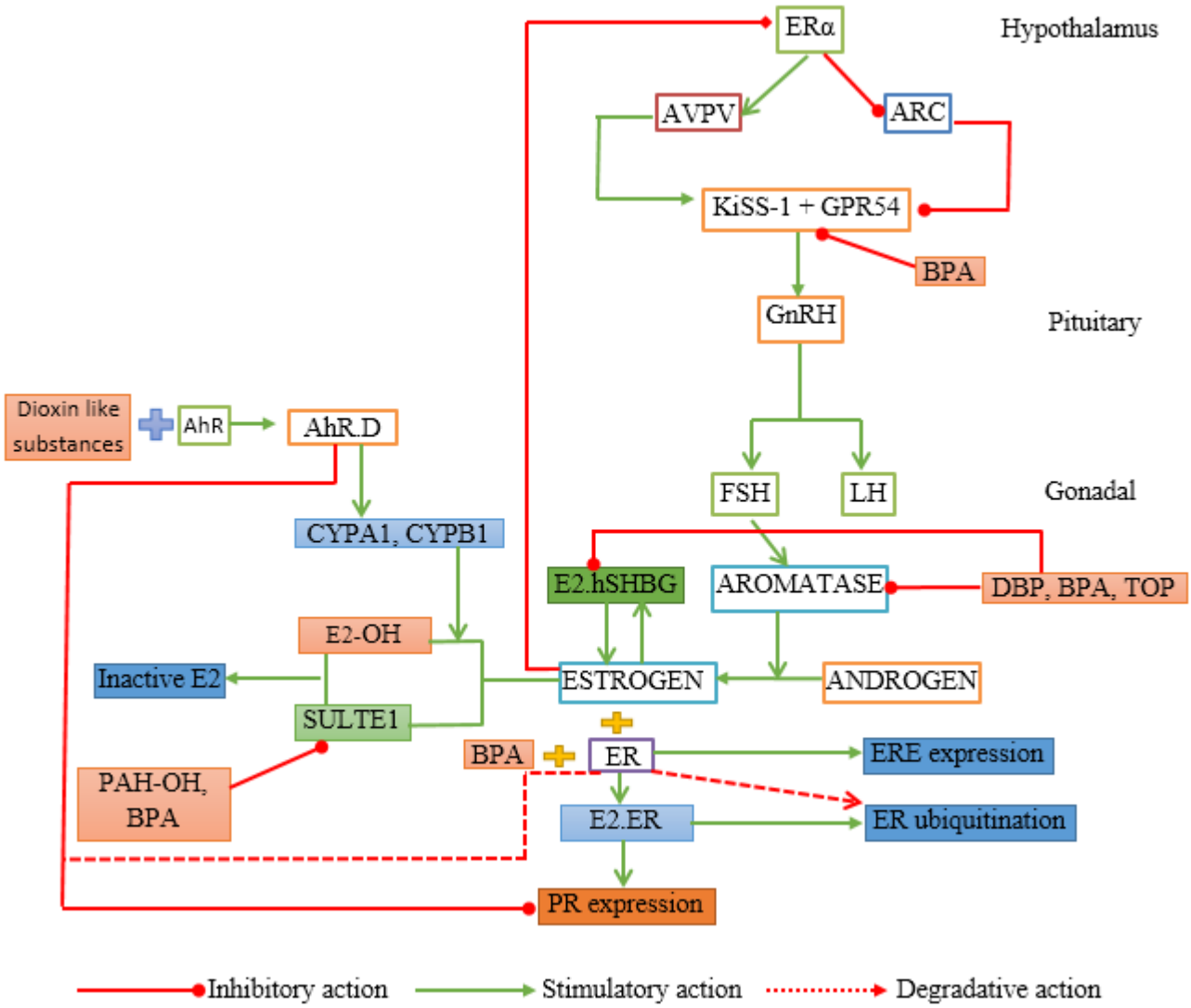


Figure 9: Schematic model for studying mixture effect in dynamic level.

This figure contains the hypothetical mixture model of characterizing risk through detail understanding of mode of chemicals' interaction with different biological components of the HPG pathways describing multiple mechanisms.