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Di-(2-ethylhexyl) Phthalate (DEHP) and Uterine Histological Characteristics

†Yong-Pil Cheon

Division of Developmental Biology and Physiology, Dept. of Biotechnology, Sungshin University, Seoul 02844, Korea

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†Corresponding author
Yong-Pil Cheon
Division of Developmental Biology and Physiology, Dept. of Biotechnology, Sungshin University, Seoul 02844, Korea.
Tel: +82-2-920-7639
Fax: +82-2-920-2736
E-mail: ypcheon@sungshin.ac.kr

ORCID
Yong-Pil Cheon
https://orcid.org/0000-0002-8497-9257

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Abstract

Phthalates have a long industrial history. It is suspected that phthalates and their metabolites have detrimental effects on reproduction and development. They are well-known for their anti-androgenic effects. Several studies have indicated that phthalates and their metabolites are reprotoxic in males and endocrine disruptors. Reproduction and embryogenesis occur in the uterus of female eutherian mammals. A horizontal analytical method is preferred to elucidate the toxic effects of phthalates on human reproduction. Nevertheless, there are vast numbers of known phthalates and not all of their modes of action have been clarified. Di-(2-ethylhexyl) phthalate (DEHP) is a commonly used plasticizer and has been the subject of numerous toxicological studies. However, few of these have reported on the toxic effects of DEHP, its metabolites, other phthalates, or mixtures on female reproduction. Acute and high doses of DEHP adversely affect uterine histology. Recently, it was disclosed that chronic exposures to low doses of DEHP have endocrine disruption efficacy. DEHP induces various cellular responses including modulation of the expression and regulation of steroid hormone receptors and transcription and paracrine factors. Uteri do not respond uniformly to DEHP exposure. The phenotypic manifestations and effects on fertility in response to DEHP and its metabolites may vary with species, developmental stage, and generation. Hence, DEHP exposure may histological alter the uterus and induce endometriosis, endometriosis, hyperplasia, myoma, and developmental and reproductive toxicity.

Keywords: Uterus, Histology, Phthalate, Di-(2-ethylhexyl) phthalate (DEHP)

Phthalates (phthalic acids) are synthetic compounds widely used in consumer products such as soft squeeze toys, waxes, paints, solvents, building materials, medical devices, electronics, personal care products, food products, and pharmaceuticals. It is predicted that ≤ 60% of the global phthalate consumption will be allocated to the aforementioned product categories by 2022 (Chemical Economics Handbook, 2018; Rowdhwal & Chen, 2018). Phthalates are alkyl esters of orthophthalic acid (o-phthalic acid). They improve the flexibility, transparency, durability, and longevity of plastics. To date, about 40 phthalates have been developed. The most frequently used phthalates include di(2-ethylhexyl) phthalate (DEHP, bis(2-ethylhexyl) phthalate), diisodecyl phthalate (DiDP), and diisononyl phthalate (DiNP) (European Union, 2007; Snejdrova & Dittrich, 2012; Wang et al., 2019). Phthalates are oily liquids with relatively low volatility. However, they form noncovalent bond with plastics and are readily released to the environment.

Phthalate do not persist in the environment as they undergo rapid biodegradation, photodegradation, and anaerobic degradation (Rudel & Perovich, 2009). Phthalates exposure occurs

mainly via ingestion (dietary sources), dermal diffusion, and inhalation (Guo et al., 2014). They are quickly metabolized to their alcohol and phthalic acid constituents (Table 1) and easily excreted (Duty & Calafat., 2005). However, they have been in use since the 1920s and are ubiquitous by now (Robbins, 2005). Hence, humans and animals have been chronically exposed to environmental phthalates. The toxicological modes of action of phthalates have been evaluated and compared for different organs and species (Kamendulis et al., 2002; Tomonari et al., 2006). Most phthalates have similar modes of action and the overall risk increases when an organism is exposed to combination of several different phthalates simultaneously. The toxic effects of phthalates also vary with developmental stage (Spencer et al., 2012). The severity of phthalate toxicity could increase with life cycle stage and long-term exposure. Certain types of phthalates have been classified as toxicants according to the 2012 United States Environmental Protection Agency (EPA) management plan. These include di-n-butyl phthalate (DBP), diisobutyl phthalate (DIBP), butyl bbenzyl phthalate (BBP), di-n-pentyl phthalate (DnPP), di-(2-ethylhexyl) phthalate (DEHP; bis (2-ethylhexyl) phthalate), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), diethyl phthalate (DEP), benzyl butyl phthalate (BzBP), and dimethyl phthalate (DMP) (Table 1). At this time, plastic manufacturers are in the process of switching to phthalate-free plasticizers.

The toxic effects of phthalates have been analyzed at the single and multiple compound levels (Hannon et al., 2015; Zhou et al., 20b; Cha et al., 2018; Kim et al., 2018; Li et al., 2020). Certain studies reported that low-molecular-weight (LMW) phthalates generally adversely affect human health at lower concentration than high-molecular-weight (HMW) phthalate. However, this observation is inconclusive (Danish, 2013; Ventrice et al., 2013). Phthalates pose a risk of reproductive toxicity for in children aged ≤ 36 mo who chew objects containing DEHP for 40 min/day. For this reason, plastic toys, child care articles, and eating vessels and utensils containing components with 1% (w/w) DEHP that are readily chewed or sucked were permanently banned for used by children aged ≤ 36 mo (Austrian Competition & Consumer Commission). DEHP may cause female infertility, sexual precocity and uterine bleeding and induce epigenetic changes during gestation. Other phthalates besides DEHP also have toxic and endocrine disrupting chemicals (EDCs) effects. In this review, however, we focused on the potential toxic effects of DEHP and other phthalates on uterine histology.

SUGGESTED EFFECTS OF PHTHALATES ON HEALTH AS ENDOCRINE DISRUPTORS

Phthalates and their metabolites may be toxicants in various animal organ systems (Silva et al., 2011; Bansal et al., 2018; Rowdhwal & Chen, 2018). They might be associated with metabolic and endocrine disorders such as insulin resistance, diabetes, and obesity. They could also be implicated in immune system dysfunction and breast cancer. However, their toxic effects on reproduction and development in humans are of greatest concern currently. Hence, several national committees (such as. ECHA and NTP-CERHA and research groups continuously evaluate the possible reproductive and developmental toxicities of phthalates (NTP Center for the Evaluation of Risks to Human Reproduction, 2003; Bansal et al., 2018; Rowdhwal & Chen, 2018; Radke et al., 2019).

Phthalates have demonstrated endocrine, testicular, ovarian, renal, nerve, liver, and heart toxicity and may induce endometriosis (Gillum et al., 2009; Rusyn & Corton 2012). In zebrafish, a mixture of DEHP and five other phthalates had LC_{50} = 0.50 ppm and caused embryo mortality and malformation. However, DEHP alone did not induce 50% mortality in zebrafish embryo even at

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Table 1. Some of the phthalates which are mostly used or known toxicant

Phthalates	Metabolites	Most used, (g/mol), CAS No.
Butyl benzyl phthalate (BBP, BBzP)[1–8]	Mono-benzyl phthalate (MBzP) Mono-butyl phthalate (MBP)	Foamed polyvinyl chloride, industrial solvent, electronics (HMW; 312.36) 85-68-7
Dicyclohexyl phthalate (1,2-benzenedicarboxylic acid dicyclohexylester) (DCHP)	Mono-cyclohexyl phthalate cyclohexanol (MCHP)	Adhesives, (HMW; 330.40) 84-61-7
Di-n-butyl phthalate (DBP, DnBP)[1–8]	Mono-n-butyl phthalate (MBP, MnBP) Mono-isobutyl phthalate (MiBP) Mono(3-carboxypropyl) phthalate (mono-Carboxyisooctyl phthalate) (MCPP)	Nail polish, adhesives, electronics, dispersions (LMW; 278.34) 84-74-2
Di-(2-ethylhexyl) phthalate (DEHP)[1–8]	Mono-(2-ethylhexyl) phthalate (mEHP) Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP, 5-oxo-MEHP) Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP; 5-OH-MEHP) Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP; 5-cx-MEPP) Mono-(2-[(carboxymethyl) hexyl] phthalate (2-cx-MMHP) Mono-(3-carboxypropyl) phthalate (mono-Carboxyisooctyl phthalate) (MCPP)	Plasticizer, polyvinyl chloride, food packages, vinyl flooring, paints, medical device, electronics (HMW; 390.56) 117-81-7
Diethyl phthalate (DEP)[7,8]	Monoethyl phthalate (mEP)	Personal care products, animal care products, electronics, dispersions (LMW; 222.24) 84-66-2
1,2-Benzenedicarboxylic acid, di-C7-11 branched and linear alkyl esters (Di-C7-11-(linear and branched)-alkyl phthalate ester; Di-711-phthalate; Di (heptyl, nonyl, undecyl) phthalate) (DHNUP)[6,7]		Plasticizer, PVC, electronics, cosmetics (HMW; 418.60) 68515-42-4
Di-isobutyl phthalate (DiBP)[4,6,7]	Mono-(carboxynonyl) phthalate (mCNP) Mono-isobutyl phthalate (mIBP)	Plasticizer, food packages, vinyl flooring, paints, electronics, dispersions (LMW; 278.34) 84-69-5
Di-isodecyl phthalate (DiDP)[1–7]	Monobutyl phthalate (mBP) Monocarboxyisononyl phthalate (MCiNP) Monohydroxyisodecyol phthalate (MHiDP) Monooxyisodecy phthalate (MOiDP) Monoisodecyl phthalate (MIDP)	Plasticizer, electronics, ear plugs (HMW; 446.66) 26761-40-0/68515-49-1
1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich (di-isoheptyl phthalate; bis(5-methylhexyl) phthalate) (DiHP, DiHeP)[7]		Plasticizer, electronics, sealant (HMW; 362.50) 71888-89-6
Di-isononyll phthaltate (DiNP)[1–3,5–7]	Mono(carboxyoctyl) phthalate (MCOP) Mono-isononyl phthalate (MINP) Mono(hydroxyisononyl) phthalate (MHINP) Mono(oxoisononyl) phthalate (MOINP)	Plasticizer, food packages, vinyl flooring, paints, eraser, electronics (HMW; 418.61) 28553-12-0/68515-48-0
Di-iso-pentyl phthalate (1,2-benzenedicarboxylic acid, 1,2-bis(3-methylbutyl) ester) (DiPP, DiPeP)[6,7]	Mono-isopentyl phthalate (MiPeP) Mono-3OH-(3-methylbutyl) phthalate (3OH-MiPeP) Mono-4OH-(3-methylbutyl) phthalate (4OH-MiPeP)	Plasticizer, PVC, electronics, propellant (HMW; 306.40) 605-50-5
Di (methoxyethyl) phthalate (bis(2-methoxyethyl) ester) (DMEP)[6,7]	Ethylene glycol menomethyl ether (EGME) Methoxyacetic acid (MAA)	Plasticizer, photographic compound, film, electronics (LMW; 282.29) 117-82-8
Dimethyl phthalate (DMP)[6–8]	Monomethyl phthalate (mMP)	Insect repellent, fluorescent products, electronics, dispersions (LMW; 194.18) 131-11-3
Di-n-hexyl phthalate (DnHP, DHP, DHEXP)[6,7]	n-Hexanol mono-n-hexyl phthalate (MHxP)	Plasticizer, electronics, footwear (HMW; 334.45) 84-75-3
Di-n-octyl phthalate (DnOP)[2,3,5,7,8]	Mono-n-octyl phthalate (mOP, MnOP) Mono-(3-carboxypropyl) phthalate (MCPP) Mono-n-heptyl phthalate (MHpP) Mono-n-pentyl phthalate (MPeP)	Plasticizer, PVC, electronics (HMW; 390.56) 117-84-0

Table 1. Continued

Phthaltates	Metabolites	Most used, (g/mol), CAS No.
Di-n-pentyl phthalate (Diamyl phthalate) (DnPP, DnPeP, DPENP, DPP)[6,7]	Mono(3-carboxypropyl) phthalate (mono-carboxyisooctyl phthalate, (MCPP) Mono(4-hydroxypentyl) phthalate (MHPP) Phthalic acid (PA)	Plasticizer, nitrocellulose, electronics, footwear (HMW; 306.38) 131-18-0
Di (2-propylheptyl) phthalate (DPHP)[6]	Mono-oxo-propylheptylphthalate (oxo-MPHP)	Plasticizer, PVC, , cable wire, roofing membranes (HMW; 446.66) 53306-54-0
1,2-Benzenedicarboxylic acid dipentylester, branched and linear (di-n-propylphthalate; dipentyl phthalate, branched and linear) (DPP)[6,7]		Plasticizer, electronics, footwear (HMW; 306.40) 84777-06-0
n-pentyl iso-pentyl phthalate (diamyl phthalate; Di-n-pentyl phthalte; 1,2-bezenedicarboxylid acid, 1,2-dipentyl ester) (PiPP, nPiPP, DnPP)[6,7]		Plasticizer, detergent, electronics (HMW; 306.40) 131-18-0

[1]California's Proposition 65 as a reproductive and developmental toxicant.

[2]California's AB1108. The bill, if passed, will ban use in the manufacture of any toy or childcare article intended for use by a child under three years of age.

[3]European Union banned as a phthalate softener in the manufacture of toys and childcare articles.

[4]European Union include to Restriction of the use of Hazardous Substances (RoHS).

[5]Japan Toy Safety Standard (ST-2002 Part3).

[6]REACH Regulation (EC)No1907/2006.

[7]Samsung.

[8]TURA, toxics use reduction act.

~ 500 ppm (Chen et al., 2014). In rats, DEHP exposure caused histopathological changes in the thyroid gland and lowered serum T4 levels (Hinton et al., 1986). In humans, MEHP, a metabolite of DEHP, alters free T4 levels (Meeker et al., 2007). DEHP and DnPeP reduced fetal rat testosterone production after uterine exposure (Veeramachaneni & Klinefelter, 2014; Howdeshell et al., 2015). DEHP induced hepatocellular and Leydig cell tumors and leukemia in rats and mice (David et al., 2000a,b; Carlson, 2010).

Phthalates cross the placental barrier. Exposure during gestation adversely affects juvenile neurodevelopment (Balalian et al., 2019). Phthalates may alter cerebral neural growth and differentiation and have negative neurocognitive and behavioral effects (Owens, 2015). DEHP exposure at 300 mg/kg BW/d and 750 mg/kg BW/d inhibited cerebellar granule precursor cell proliferation in male offspring and impaired neuromotor development through Shh signaling (Fu et al., 2019). In male rat progeny, DnPP, DnPeP (at high oral dose during pregnancy), or DEHP mixtures caused reproductive tract malformations such as hypospadias, cryptorchidism, and anogenital distance (Hauser & Calafat, 2005; Howdeshell et al., 2008). During the first trimester, DEHP metabolites cause malformations of the juvenile male reproductive tract (Watkins et al., 2017). Exposure to DEHP or its metabolites during the first trimester is correlated with increases in serum 17 β -estradiol (E2) in males aged 8–14 y (Watkins et al., 2017). DEHP exposure increases insulin resistance by creating imbalances between oxidative stress and antioxidant defenses. DEHP induces oxidative stress in the pancreas (Kim et al., 2013). The negative effects of DEHP exposure on the immune system are more severe during pregnancy than they are at later developmental stages (Holladay & Smialowicz, 2000). Mono-carboxyisooctyl phthalate (MCPP) is a metabolite of several HMW phthalates and dibutyl phthalate. It is associated with an elevated risk of asthma (Berger et al., 2019). EDC exposure during gestation results in postnatal metabolic disorders and adverse health effects (Lee et al., 2017). DEHP (100 μM) enhanced adipogenic differentiation in murine mesenchymal stem cells (Biemann et al., 2012).

Phthalate reprotoxicicity was manifested as phthalate syndrome in male rodents and preterm

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birth in female rodents (Howdeshell et al., 2008; Ferguson et al., 2014; Hannon and Flaws, 2015). Urinary phthalate metabolite concentrations have been associated with spontaneous abortion (miscarriage) and preterm birth (Fromme, 2013; Ferguson et al., 2014). The toxic effects of phthalates may be both transgenerational and ontogenic (Walker, 2011; Zhou et al., 2017). Therefore, reprotoxic phthalates are banned in the EU and other countries (Danish, 2013). Moreover, the concentrations of reprotoxic phthalates in products are limited by law in the United States (Pak & McCaulery, 2007), South Korea, and other countries (Cho & Lee, 2018). DEHP, DnBP, DiBP, BBzP, DnPeP, DiPeP, DHNUP, DnHP, and DMEP are classified as category 1B reproductive toxicants under Annex VI to the Classification. Labelling, and Packaging (CLP) Regulation (EC 1272/2008) (HBM4EU). This statute also defined the tolerable daily intake (TDI) of these substances (Lyche et al., 2009).

One aspect of phthalate toxicity is endocrine disruption. The National Institute of Environmental Health Sciences defines EDCs as "chemicals that interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological and immune effects." Phthalates are broadly classified as endocrine disruptors in wildlife and human (Hauser & Calafat, 2005; Lyche et al., 2009; Su et al., 2014; Bansal et al. 2018; Zamkowska et al., 2018). In humans, phthalate exposure alters steroid levels during gestation, infancy, and adulthood (Hauser & Calafat, 2005; Lyche et al., 2009; Su et al., 2014). Laboratory animals subjected to EDCs variously presented with early onset of puberty, hindrance of male or female reproductive tract development, interference with the natural functioning of the hormone system, reproductive and genital defects, low adolescent male testosterone, low adult male sperm counts, and impaired uterine development (Agarwal et al., 1986; Dalgaard et al., 2001; Spencer et al., 2012).

DEHP is ubiquitous in the environment. It can be found in the air, water, and soil. It was detected in poultry, cooking oils, and cream-based dairy products (Serrano et al., 2014). A report of the 'Monte Carlo Risk Assessment' program (MCRA 7.0) indicated the concentrations of DEHP and DiBP were the highest and second highest of the phthalates measured in 550 food products sold on the Belgian market. The consumption of bread contributed to 31.4% of the total dietary phthalate intake in the general adult population (Sioen et al., 2012). The human body rapidly metabolizes ingested DEHP to MEHP and transforms the latter to 5-OH-MEHP, 5-oxo-MEHP, 5-cx-MEPP, and 2-cx-MMHP (Table 1) (Serrano et al., 2014). DEHP and its metabolites are generally regarded as EDCs and have adverse health effects in at all life stages. The reproductive organs are the main targets of EDC (Hannon et al., 2015). DEHP and its metabolites are generally regarded as EDCs and have adverse health effects at all life stages. The reproductive organs are the main targets of EDCs (Hannon et al., 2015). DEHP and its metabolites bind to estrogen receptors (ERs) and induce ER expression (Cavanagh et al., 2018). In adult males, the main DEHP target is the testis. DEHP causes testicular infertility (Hu et al., 2009). It reduces sperm quality, causes testicular atrophy, and induce Sertoli cell vacuolation and hypospermatogenesis (Hauser, 2006; Street et al., 2018). DEHP downregulates steroidogenic acute regulation protein (StAR) and *in utero* fetal testicular 17 α -hydroxylase and cytochrome P450 17A1 mRNAs by direct action on the testis (Kariyazono et al., 2015). Male rats exposed via placental diffusion to DEHP at a dosage range of 100–750 mg/kg BW/d present with diminished mineralocorticoid receptor expression in the Leydig cells and reduced testosterone levels (Martinez-Arguelles et al., 2009, 2011).

The effects of phthalates on female reproduction are controversial Certain reports suggested that phthalate exposure is correlated with female infertility. Caserta et al. (2013) reported the blood levels of the EDCs whose effects on female reproductive health were evaluated. In rats, DEHP altered the hypothalamus-pituitary-ovary axis and gonadotropin and sex steroid hormone expression (Hirosawa et al., 2006; Ma et al., 2006, 2011; Liu et al., 2014). Estrogenic DEHP

reduced estrogen levels and inhibited follicle growth *in vitro* (Kalo et al., 2015) It also diminished primordial follicle recruitment in rodents (Hannon et al., 2014). DEHP administration (2 g/kg BW/d) lowered serum E2. Its metabolites prolonged estrus cycles and suppressed E2 production in granulosa cells vis a receptor-mediated signaling pathways (Lovekamp-Swan & Davis, 2003). Chronic low-dose DEHP in drinking water induces changes in tissue layer thickness, ER and PR expression, and tissue-specific ER and PR localization (Kim et al., 2018). DEHP treatment alters endometrial epithelial cell proliferation and morphology (Somasundaram et al., 2016). EDCs may directly or indirectly modify endometrial responses to steroid hormones and promote endometriosis (Rier et al., 2002). Certain patients with endometriosis presented with significantly higher DEHP levels than those without it (Cobellis et al., 2003; Reddy et al., 2006; Kim et al., 2011). Caserta et al (2013) reported high blood levels of MEHP, PFOS, and bisphenol-A (BPA) in females presenting with endometriosis. Hence, estrogenic EDCs such as DEHP may cause endometriosis, uterine fibroids, fetal growth restriction, and pregnancy loss (Spencer et al., 2012; Kim et al., 2017).

Phthalates are usually detected along with several of their metabolites in human fluid samples (Jensen et al., 2015). Prenatal phthalate exposure may have multigenerational and transgenerational effects on female reproduction inducing increases in uterine weight, body weight and decreases anogenital distance. Phthalate exposure increased of the number of cystic ovaries in F1 and F2 females. In F3 females, it increased uterine weight and decreased anogenital distance. It also caused fertility complications in F1 and F3 females (Zhou et al., 2017; Li et al., 2020). EDC exposures might increase the risk of breast cancer induction (Maskarinec & Noh, 2004). The co-relation among DEHP, its metabolites, and breast cancers are controversial (Ahern et al., 2019; Morgan et al., 2017). However, DEHP and its metabolites have strong affinities for progesterone receptor (PR) and there may 82%–95% overlap between PR-interacting residues. Thus, DEHP and its metabolites could disrupt normal PR signaling which results in negative reproductive effects (Sheikh et al., 2016). In T-47D breast cancer cells, DEHP and MEHP induced the PR α expression and nuclear localization. Phthalate treatment caused T-47D cell proliferation without apoptosis (Crobeddu et al., 2019). On the other hand, DEHP enhanced ER α -mediated transcriptional activity and decreased ER α protein levels in hypoxic in MCF-7 breast cancer cells (Park et al., 2019).

As DEHP, BBzP, DnBP, and DiBP are endocrine disruptor, they are categorized as substances of very high concern (SVHC) and have been candidates for inclusion in Annex XIV of the REACH regulation since 2017 (HBM4EU). Phthalates are most likely to cause abnormalities in the reproductive systems of animals following exposures during gestation and infancy (Hauser & Calafat, 2005; Lyche et al., 2009; Su et al., 2014). Female infertility may be associated with EDC exposure. DEHP is a common EDC and classified as a harmful chemical (Wang et al., 2019). Exposure to DEHP increases the bioavailability of other EDCs such as BPA because these agents compete for the same metabolic enzymes (Borman et al., 2017). The uterus is a key organ in mammalian development and the main target organ of EDCs. Furthermore, uterine cells respond to estrogen and progesterone. Until now, data on the possible effects of DEHP on the uterus were very limited. In the next section, we address the effects of DEHP on uterine histology.

DEHP AND UTERINE HISTOLOGY

Harmonization of the functions of heterogeneous uterine cell types and maintenance of reproductive system homeostasis are vital to successful implantation and reproduction. The uterus comprises luminal and glandular epithelial, stromal, smooth muscle, and uterine-specific or uterine-

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nonspecific immune cells. Harmonization of cell function under the control of the correct levels of sex steroid hormones prepares the uterine environment for normal physiological responses (Cheon et al., 2002). Uterine histology is species-specific and adapted to reproduction patterns. In rodents, Müllerian ducts form bipartite uteri with two horns and two cervical oses.

During radial patterning, uterine morphogenesis establishes the endometrium, myometrium (middle muscular layer consisting of circularly arranged inner and longitudinally arranged outer smooth muscle layers), and perimetrium. The morphogenic processes that occur during the postnatal period include endometrial stroma organization and stratification, myometrium differentiation and growth, and coordinated endometrial gland development. In most eutherian mammals, postnatal endometrial and glandular morphogenesis occurs in species-specific placentation patterns and reproduction patterns. The functional layer of the endometrium is established for implantation and post differentiation (Walker, 2011; Spencer et al., 2012).

Uterine maturation is regulated by estrogen and progesterone. These hormones have specific receptors (ER and PR) that function in the uterus as transcription factors (Okada et al., 2005). ER α predominantes in all uterine cell types. ER β is expressed at relatively lower expression levels in the uterus and is confined mainly to the subepithelial stromal cells (Wang et al., 2000). PR is strongly localized to the epithelial cells during the diestrus stage and to the stroma during the proestus stage (Ohta et al, 1993; Tan et al., 1999). ERs and PRs are maintained in an inactive state by being bound to an inhibitory protein complex containing heat-shock protein 90 (Hsp90). They are then activated by the binding of estrogen and progesterone (Nardulli & Shapiro, 1993; Klinge, 2001). Uterine wet weight and volume increase under the influence of estrogen (Shelby et al., 1996). Estrogen promotes endometrial cell proliferation and growth and increases vascular permeability. Progesterone reduces ER levels and promotes cell differentiation and angiogenesis (Ma et al., 2001).

The endometrium undergoes substantial changes during the reproductive cycle. It is composed of surface epithelium, endometrial glands, and the lamina propria. The endometrial cells express ERs and PRs under the regulation of steroid hormones. The luminal and glandular epithelial cells form simple cuboidal or columnar epithelial tissues (Li & Davis, 2007; Spencer et al., 2012). In rodents, the luminal epithelial, stromal, and myometrial cells proliferate during proestrus, the luminal and glandular epithelial cells are cuboidal, and apoptotic epithelial and stromal cells may be observed (Marcus, 1974; Dharma et al., 2001). During estrus, the luminal epithelial cells differentiate from cuboidal to columnar and develop large cytoplasmic volume. Vacuolar degeneration and apoptosis may be observed in the luminal and glandular epithelia as the uterus reorganizes and prepares for implantation. Apoptotic luminal epithelial cell are observed at this time (Dharma et al., 2001). During metestrus, the epithelial cells are large and columnar but their cytoplasmic volumes are substantially reduced. Glandular apoptosis is relatively less frequent and there are comparatively fewer inflammatory cells in the lamina propria. Apoptotic luminal epithelial, glandular, and stromal cells may be observed (Dharma et al., 2001). During early diestrus, the glandular, stromal, and vascular cells proliferate and increase the thickness of the uterine endometrium. The luminal epithelial cells organize into a single layer of tall columnar cell with basal nuclei. Apoptotic stromal cells can be seen (Dharma et al., 2001).

Estrogen and progesterone tightly regulate endometrial cell proliferation and differentiation (Cheon et al., 2002; Singh et al., 2011). The adverse effects of high estrogen dose are well documented. Estrogen exposure during the perinatal period induce cystic endometrial hyperplasia, squamous metaplasia, adenomyosis, and myometrial and general uterine hypoplasia (Houston et al., 2003). ER α overexpression increases the number of apoptotic cells in the endometrial epithelium and decreases the number of implantation sites (Tomic et al., 2007). It is postulated that estrogenic EDCs have nearly the same effects on the uterus as endogenous estrogen itself. DEHP inhibits E2

binding to its receptors (Jobling et al., 1995) and induces estrogenic activity via ERs (Cavanagh et al., 2018). The mode of action of DEHP differs among species. Tomonari and colleagues (2006) reported that exposure to 0–100 mg/kg BW/d in marmosets from weaning (3 mo) to sexual maturity (18 mo) did not cause abnormal histological changes. In rats, 1,000 mg/kg BW/d DEHP exposure during gestation days 6–15 reduced uterine weight (Hellwig et al., 1997; Ambe et al., 2019). In contrast, 0–100 mg/kg BW/d DEHP exposure for 30 d did not have that effect (Somasundaram et al., 2016). Uterine weight was increased by DEHP exposure in drinking water at a concentration of 133 μg/L (Kim et al., 2018).

Uterine horns develop after birth and form an external myometrium surrounding the mesenchymal compartment (Cunha 1976; Kurita, 2011). In rats, DEHP treatment reduced the relative uterine horn diameter (Somasundaram et al., 2016). In endometrial cells, DEHP exposure enhanced comparative MMP-2 and MMP-9 activity, cellular invasiveness, Erk phosporylation, and p21-activated kinase 4 expression (Kim et al., 2015). Uterine gland development is vital to conceptus development in placental mammals (Burton et al., 2002; Gray et al. 2002). Interactions between the epithelium and the stroma in the gland-forming area are critical. They are mediated by steroid hormones, paracrine factors, and the networks that link them (Lubahn et al., 1993; Gray et al., 2000; Taylor et al., 2001; Carpenter et al. 2003; Mericskay et al., 2004; Jeong et al., 2010). The adverse effects of DEHP on uterine gland development and function are controversial. Somasundaram et al (2016) suggested that DEHP treatment lowers the number of endometrial glands and disrupt their structure. However, other researchers reported the opposite results. Richardson et al (2018) showed that 200 mg/kg BW/d DEHP exposure for 30 d increased the relative number of endometrial glands. However, it is generally believed that DEHP has toxic effects on the uterus (Kim et al., 2018; Ambe et al., 2019). Chronic low-dose exposure to DEHP induces histological changes. A concentration of 50 μg/L DEHP in drinking water increased the relative number of endometrial glands (Kim et al., 2018).

The uterine mesenchyme directs and specifies the surfacing epithelium. It also organizes the endometrial stroma and promotes myometrial differentiation (Cunha 1976, 1989; Kurita et al., 2001). Intrinsic growth factor systems and the ECM microenvironment mediate epithelial-mesenchymal interactions (Spencer et al., 1993; Hu et al., 2004). DEHP treatment at 200 μg/kg BW/d for 30 d reduced uterine epithelial cell proliferation (Richardson et al., 2018). Thinning of the uterine layer was observed in rats treated with 0–100 mg/kg BW/d DEHP for 30 day (Somasundaram et al., 2016). However, chronic low-dose DEHP treatment (133 μg/L and 1,330 μg/L in drinking water) increase endometrial thickness in mice (Kim et al., 2018). On the other hand, DEHP may induce myometrial cell proliferation. Chronic administration of 133 μg/L DEHP in drinking water increased relative myometrial thickness (Kim et al., 2018).

Even individual phthalate compounds can influence uterine histology. However, recent studies revealed that phthalate mixtures had similar effects on the uterus. Exposure to an environmentally relevant phthalate mixture (35% DEP, 21% DEHP, 15% DBP, 15% DiNP, 8% DiBP, and 5% BBP) during gestation caused multigenerational alterations in uterine histology (Li et al., 2020). Nevertheless, Li et al (2020) reported that phthalate mixtures did not affect uterine wet weight, endometrium size, number of glands, or inner or outer myometrium thickness in any generation. However, luminal epithelial cell proliferation decreased in the F1 generation in response to phthalate mixtures. In F1 subjected to 200 μg/kg/d phthalate mixture, 0.05% of their luminal epithelial cells were Ki67-positive. Luminal epithelial cell proliferation increased in the F2 generation and 62.88% of those cells were Ki67-positive. For all generations, the phthalate mixture increased the relative amount of multilayered luminal epithelia. Phthalate mixture exposure during gestation resulted in the formation of large, dilated endometrial glands in all generations. In certain

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animals, these glands penetrated the myometrial layer (Li et al., 2020).

It is uncertain whether DEHP causes female infertility. In an animal model, high dose of DEHP impaired female fertility (Schmidt et al., 2012). Recently, however, we investigated the possible that chronic exposures to low-dose DEHP have EDC efficacy in the uterus. However, this treatment did not diminish parental or F1 fertility (Cha et al., 2018; Kim et al., 2018). For women receiving IVF/ICSI treatment, urinary DEHP metabolite levels were not correlated with clinical outcomes in the total population (Hauser et al., 2016; Deng et al., 2020).

DEHP may also induce various cellular responses. At the cellular level, DEHP increases endometrial ER α and PR proteins levels (Somasundaram et al., 2016). DEHP can induce oxidative stress in endometrial stroma cells (Cho et al., 2015). DEHP decreased ERa protein expression in Ishikawa human endometrial adenocarcinoma cells and slightly decreased hypoxic VEGF secretion (Park et al., 2019). DEHP enhanced myometrial and leiomyomal cell proliferation and antiapoptosis (Kim et al., 2017). DEHP is agonistic to PR (Sheikh et al. 2016). DEHP upregulated uterine GnRH receptors and hypothalamic GnRH in pubertal rats (Liu et al., 2016). DEHP induces inflammation mediating peroxisome proliferator-activated receptor gamma (PPAR γ) in cultured primary endometrial stroma cells without epithelial-mesenchymal transition (EMT) (Huang et al., 2016).

Estrogen and progesterone have the opposite effects in the paracrine maintenance of uterine histology (Li et al., 2011; Chung et al., 2015). The complexity of steroid hormonal regulation and the roles of various cells in uterine function render it difficult to elucidate the potential effects of DEHP on uterine histology. EDCs may have detrimental effects on reproductive health. Further studies are required to clarify the mechanisms of EDC toxicity in animal models and humans. DEHP causes abnormal histological phenotypes and their associated aberrant cellular responses may play roles in uterine pathogenesis of uterus.

CONCLUSION

Phthalates have diverse and useful industrial applications. However, certain ubiquitous phthalates such as DEHP are toxic to mammals and the environment. Therefore, numerous international governments have restricted or even banned the use of phthalates. Polyethylene, polypropylen, polyurethane, silicone, and ethylene vinyl acetate have substituted for phthalates in plastic fabrication. Nevertheless, long-term exposure to low levels of residual DEHP and its metabolites such as MEHP may cause endocrine disruption and alter uterine histology and sex hormone receptors in mammals.

EDC exposure at critical period of differentiation in the female reproductive tract might change developmental programming and profoundly influence the outcome of functional reproductive disorders (Norgil Damgaard et al., 2002; Masse et al., 2009; Walker et al., 2011). In model animals, prenatal EDC exposure results in uterine lesions such as altered steroid receptor concentration and responsiveness, persistent gene induction or repression, cystic endometrial hyperplasia, squamous metaplasia, and myometrial and general uterine hypoplasia (Spencer et al., 2012).

DEHP adversely affects adult female uterine histology. DEHP induces various cellular responses such as alteration of the expression and regulation of steroid hormone receptors and transcription factors. However, whether DEHP decreases fertility remains inconclusive. Recent research suggested that like acute and high-concentration DEHP exposures, chronic-low-dose DEHP and environmentally relevant phthalate mixture exposure may modify uterine histology and induce reproductive diseases such as endometriosis, hyperplasia, and myoma.

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