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

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Abstract

Phthalates and those metabolites have long history in industry and suspected to have deficient effects in development and reproduction. These are well-known anti-androgenic chemicals and many studies have examined the effects of these compounds on male reproduction as toxins and endocrine disruptors. Uterus is a key organ for proper embryo development, successful reproduction, and health of eutherian mammals including women. To understand the effects of the phthalate, the horizontal approach with a whole group of phthalate is best but the known phthalates are huge and all is not uncovered. Di-(2-ethylhexyl) phthalate (DEHP) is the most common product of plasticizers in polymer products and studied many groups. Although, there is limited studies on the effects of phthalates on the female, a few studies have proved the endocrine disrupting characters of DEHP or phthalate mixture in female. An acute and high dose of DEHP has adverse effects on uterine histological characters. Recently, it has been revealed that a chronical low-dose exposing of DEHP works as endocrine disrupting chemicals (EDC). DEHP can induce various cellular responses including the expression regulation of steroid hormone receptors, transcription factors, and paracrine factors. Interestingly, the response of uterus to DEHP is not monotonous and the exposed female has various phenotypes in fertility. These suggest that the exposing of DEHP may causes of histological modification in uterus and of disease in female such as endometriosis, hyperplasia, and myoma in addition to developmental and reproductive toxicity.

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

Phthalates (phthalic acids) are developed chemical compounds to be used widely in the manufacture of plastic consumer products such as soft squeeze toys, waxes, paints, solvents, building materials, medical devices, modern electronics, personal care products, food products, and pharmaceuticals forecasted 60% of world consumption in 2022 (IHS Markit, 2018; Rowdhwal & Chen, 2018). Phthalates are esters of orthophthalic acid (o-phthalic acid) with alkyl chains. They increase flexibility, transparency, durability, and longevity of plastics, and very useful. So far, about 40 phthalates are developed and the mostly used phthalates include di (2-ethylhexyl) phthalate (DEHP, bis [2-ethylhexyl] phthalate), di-isodecyl phthalate (DiDP), di-isononyl phthalate (DiNP) (European Chemicals Bureau, 2007; Snejdrova & Dittrich, 2012; Wang et al., 2019). Phthalates are oily liquids and do not evaporate easily. On the other hand, phthalates bind to plastics in nonconvalent bond and easily release to the environment.

Phthalates do not persist due to rapid biodegradation, photodegradation, and anaerobic degradation

(Rudel & Perovich, 2009). Exposing to phthalates occurs mainly via ingestion (dietary sources), dermal diffusion, and inhalation (Guo et al., 2014). They are quickly converted to the metabolites through forming the respective alcohols and phthalic acid (Table 1) and easily excreted (Duty et al., 2005). However, phthalates have been used since 1920s and the amount of plasticized plastics are huge and ubiquity (Robbins, 2005). So they are exposed chronically to human and other lives. The action modes for toxicant in organs and for species differences have been evaluated (Kamendulis et al., 2002; Tomonari et al., 2006). The facts that most of the phthalates have similar modes of action and the overall risk increases by exposing to the combined phthalates are big issue. On the other hand, the effects of the toxicant show the developmental stage specific manners (Spencer et al., 2012), so their adverse effects become more sever by the exposing time at life cycle stages. Therefore, by the environmental awareness and perceptions for toxicity, some types of them are classified as toxicant in U. S. Environmental Protection Agency's (EPA's) current management plan (at 2012) and others: di-n-butyl phthalate (DBP), diisobutyl phthalate (DIBP), butyl benzyl phthalate (BBP), di-n-pentyl phthalate (DnPP), di-(2-ethylhexyl) phthalate (DEHP; bis (2-ethylhexyl) phthalate), di-n-octyl phthalate (DnOP), DINP, DIDP, diethyl phthaltate (DEP), benzylbuthyl phthalate (BzBP), and dimethyl phthalate (DMP) (Table 1). Now, phthalate-free plasticizers become as transition in plastic manufactures.

The toxic effects of phthalates on health have been studied in complex or single compound levels (Hannon et al., 2015; Zhou et al., 2017; Cha et al., 2018; Kim et al., 2018; Li et al., 2020). In some document, low-molecular-weight phthalates generally have effects on human health at lower concentration than the group of high-molecular-weight. However, it is difficult to define the conclusion (Danish, 2013; Ventrice et al., 2013). It is clear that phthalates have a risk of reproductive toxicity for children up to 36 months of age, if they were to chew objects containing DEHP for more than 40 minutes per day. So, there is a permanent ban on plastic toys, childcare articles, and eating vessels and utensils intended for children aged up to 36 months that contain or have an accessible component containing more than 1% by weight of DEHP, are readily chewed and or sucked (Austrian Competition & Consumer Commission). It has been suggested that DEHP can cause female diseases such as infertility, sexual precocity and uterine bleeding. Besides, the epigenetic changes can be induced during gestation. Although other phthalates also have toxicity and endocrine disrupting chemicals (EDCs) effects, in here, the possible effects of phthalates especially DEHP and the uterine histology are the main keywords.

SUGGESTED EFFECTS OF PHTHALATES ON HEALTH AS ENDOCRINE DISRUPTORS

Phthalates and their metabolites can cause of toxicant in various main organ system of animals (Silva et al., 2011; Bansal et al., 2018; Rowdhwal & Chen, 2018). It is suspected that phthalates are associated with metabolic and endocrine problems such as insulin resistance and diabetes, and obesity. It also has a relationship with disease such as immune problems and breast cancer. Although such effects are big issues, the effects on the reproduction and development are the most important things for human being. Based on the suggested adverse effects, many national committee (e.g. ECHA, NTP-CERHA) and research groups have continuously evaluated the potential 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).

The adverse effects of phthalates on organs and organ systems have a wide scope including endocrine toxicity, testicular toxicity, ovarian toxicity, endometriosis, renal toxicity, neurotoxicity,

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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.

hepatotoxicity, cardiotoxicity (Gillum et al., 2009; Rusyn & Corton, 2012). In zebafish, DEHP and five phthalate mixture is toxic with an LC_{50} of 0.50 ppm, and causes of embryonic mortality and malformation. DEHP does not induced 50% death of zebrafish embryo even at high (about 500 ppm) concentration (Chen et al., 2014). In rat, DEHP exposing can be a cause of histopathological changes of thyroid gland and decreases of T4 levels (Hinton et al., 1986). MEHP, a metabolite of DEHP, alter the free T4 levels in man (Meeker et al., 2007). DEHP and DnPeP have potent for reducing fetal rat testosterone production by the utero exposing (Veeramachaneni & Klinefelter, 2014; Howdeshell et al., 2015). DEHP induces tumors such as hepatocellular tumors, leukemia, and Leydig cell tumors in rats and mice (David et al., 2000; Carlson, 2010).

Phthalates can cross the placenta. Exposing during gestation has adverse effects on child neurodevelopment (Balalian et al., 2019). It may induce alterations in brain neural growth and differentiation with neurocognitive and behavioral consequences (Owens, 2015). The exposure to 300 and 750 mg/kg/d DEHP inhibits the proliferation of cerebellar granule cell precursor cells in male offspring and ultimately results in impairment of neuromotor development through Shh signaling (Fu et al., 2019). DnPP (DnPeP, high dose oral exposure during pregnancy) or DEHP mixture in rat acts as a toxicant in reproductive tract malformation of male offspring, such as hypospadias, cryptorchidism, and shortened anogenital distance (Hauser & Calafat, 2005; Howdeshell et al., 2008). DEHP metabolites during first trimesters cause malformation in male offspring's reproductive tract (Watkins et al., 2017). Exposing DEHP or its metabolites in the first trimester has relationship with the increased serum estradiol levels in males at 8–14 years of age (Watkins et al., 2017). Insulin resistance is increased after DEHP exposure due to an imbalance between oxidative stress production and antioxidant defenses. DEHP induce oxidative stress in pancreas (Kim et al., 2013). Exposure affects to DEHP during pregnancy on immune system is more greatly than later exposures (Holladay & Smialowicz, 2000). Mono-carboxyisooctyl phthalate (MCPP), a metabolite of several high molecular weight phthalates and dibutyl phthalate is associated with increased odds for asthma (Berger et al., 2019). The exposing EDCs during

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gestation cause the metabolic disorders and adverse health effects after birth (Lee et al., 2017). Rising obesity rates are one of the serious problems world widely. Phthalates are supposed to disturb metabolism. Interestingly, DEHP (100 μM) enhances adipogenic differentiation of murine mesenchymal stem cells (Biemann et al., 2012).

Reprotoxic characters of phthalates are shown in both males and females such as phthalate syndrome in male rodents and preterm birth (Howdeshell et al., 2008; Ferguson et al., 2014; Hannon and Flaws, 2015). Urinary concentration of their metabolites has been associated with pregnancy loss and preterm birth (Fromme, 2013; Ferguson et al., 2014). It has been evaluated that the toxic effects of phthalates have both transgenerational and ontogenic effects (Walker, 2011; Zhou et al., 2017). Therefore, reprotoxic phthalates are not allowed to be placed on the market in EU and other countries (Danish, 2013). In addition, their concentration in products is limited by law in United States (Pak & McCaulery, 2007), Korea and other countries (Cho & Lee, 2018). DEHP, DnBP, DiBP, BBzP, DnPeP, DiPeP, DHNUP, DnHP, and DMEP are classified as reproductive toxicants category 1B under Annex VI to the classification, labelling and packaging (CLP) regulation (EC 1272/2008) (HBM4EU, 2020). The tolerable daily intake (TDI) is defined to them (Lyche et al., 2009).

One of the reasons of toxicity is the character of them as endocrine disrupting chemicals. As we know, 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 disruptor in the wildlife and human (Hauser & Calafat, 2005; Lyche et al., 2009; Su et al., 2014; Bansal et al., 2018; Zamkowska et al., 2018). Phthalate exposure alters steroid levels and not only works in adults but also during gestation and infant period in human (Hauser & Calafat, 2005; Lyche et al., 2009; Su et al., 2014). In lab animals, the evaluated possible roles are followed: early onset of puberty, interfering with the male or female reproductive tract development, interfering with the natural functioning of the hormone system, causing reproductive and genital defects, lower testosterone levels in adolescent males, lower sperm count in adult males, and disrupted uterine development (Agarwal et al., 1986; Dalgaard et al., 2001; Spencer et al., 2012).

DEHP is ubiquity in the environment (air, water, and soil). Besides, it can be found in poultry, cooking oils and cream-based dairy products (Serrano et al., 2014). According to the report of 'Monte Carlo Risk Assessment' program (MCRA 7.0), the intake concentration of DEHP is the highest one and followed by DiBP among the 550 food products sold on the Belgian market. Bread contributes to the total exposure at rate of 31.4% in the general adult population (Sioen et al., 2012). After importing to the body, our body system rapidly metabolizes to MEHP and then further metabolized to 5-OH-MEHP, 5-oxo-MEHP, 5-cx-MEPP, and 2-cx-MMHP as shown in Table 1 (Serrano et al., 2014). DEHP and its metabolites are generally considered as EDC and have adverse effects on health in all life stage. The reproductive organs are main EDC target organs (Hannon et al., 2015). DEHP and its metabolites can bind to estrogen receptors (ERs) and induce the expression of ERs (Cavanagh et al., 2018). In adult male, the main target for DEHP is testis and DEHP causes of testicular infertility (Hu et al., 2009). DEHP leads to lower sperm quality, testicular atrophy, Sertoli cell vacuolation and hypostermatogenesis (Hauser, 2006; Street et al., 2018). DEHP decreases the expression of steroidogenic acute regulation protein (StAR) mRNA and lowers the *in utero* fetal testicular mRNA levels of 17 α -hydroxylase and cytochrome P450 17A1 through directly working on testis (Kariyazono et al., 2015). DEHP-exposed male rat through placental diffusion during gestation (100–750 mg/kg bw/day) diminishes mineralocorticoid receptor expression in Leydig cell and testosterone level (Martinez-Arguelles et al., 2009, 2011).

The effects of phthalates on female reproduction are controversy, but some reports suggested the prospection of some phthalates have correlation with female infertility. Caserta et al. (2013) reported the blood level of EDCs to evaluate the women's reproductive health. In rat, the treatment of DEHP affects hypothalamus-pituitary-ovary axis and the expression of gonadotropins and sex steroid hormones (Hirosawa et al., 2006; Ma et al., 2006, 2011; Liu et al., 2014). Estrogenic DEHP can reduce the estrogen levels *in vitro* and inhibits *in vitro* follicle growth (Kalo et al., 2015) and primordial follicle recruitment in rodents (Hannon et al., 2014). DEHP (2 g/kg BW) administration causes the decrease of serum 17 β -estradiol level and the prolonged estrous cycles through it metabolites and a receptor-mediated signaling pathway to suppress 17 β -estradiol production in granulosa cells (Lovekamp-Swan & Davis, 2003). Chronic low-dose DEHP in drinking water induces the change of the thickness of tissue layers, ERs and PRs expression levels, and tissue specific localization patterns of ERs and PRs (Kim et al., 2018). DEHP treatment effects on the endometrial epithelial cell proliferation and morphology (Somasundaram & Manokaran, 2016). It suggested that EDCs directly or indirectly affect the endometrial response to the steroid hormones and can lead endometriosis (Rier & Foster, 2002). The patients having endometriosis have significantly higher levels of DEHP compared with women without endometriosis (Cobellis et al., 2003; Reddy et al., 2006; Kim et al., 2011). Caserta et al. (2013) reported the high blood levels of MEHP, PFOS, and BPA in endometriosis group. So, it is suggested that estrogenic EDCs-like DEHP effects on endometriosis, uterine fibroids, fetal growth restriction, and pregnancy loss (Spencer et al., 2012; Kim et al., 2017).

Usually phthalates are detected in human fluid samples with multiple phthalate metabolites (Jensen et al., 2015). Prenatal phthalate mixture exposure induces multigenerational and transgenerational effect on female reproduction. It causes an increase of the uterine weight, anogenital distance, and body weight. In addition, it causes an increase of the number of cystic ovaries in F1 and F2 female. In the case of F3 female, it causes the increases uterine weight, decreased anogenital distance, and fertility complications in the F1 and F3 female (Zhou et al., 2017; Li et al., 2020). It is suggested that breast cancer can be induced more easily by the exposing to EDCs (Maskarinec & Noh, 2004). So far, the co-relationship between DEHP and its metabolites and breast cancers are controversy (Morgan et al., 2017; Ahern et al., 2019). However, interestingly, DEHP and its metabolites have interaction with progesterone receptor (PR) with high affinity and overlapping (82%–95%) between PR interacting residues. It suggests that their disrupting potential in normal PR signaling, resulting in adverse reproductive effects (Sheikh et al., 2016). In breast cancer cell, T-47D, DEHP and its metabolite MEHP induce the PR α expression and nuclear localization. Their exposing result in the proliferation of T-47D cell without apoptosis (Crobeddu et al., 2019). On the other hand, DEHP can also enhance ER α -mediate transcriptional activity under hypoxia and decrease ER α protein levels under hypoxia in MCF-7 cells, a breast cancer cell line (Park et al., 2019).

Because of endocrine disrupting property of DEHP, BBzP, DnBP, and DiBP, these compounds are identified as substances of very high concern (SVHC) and included in the candidate list for the inclusion in Annex XIV of the REACH regulation since 2017 (HBM4EU, 2020). Phthalates cause abnormalities in the reproductive systems of animals with the greatest effects when they are exposed during gestation and infant period (Hauser & Calafat, 2005; Lyche et al., 2009; Su et al., 2014). It is considered that infertility in women's reproductive health may be concerned with the EDCs. Among phthalates, many groups have been studies with DEHP. As discussed previously, DEHP is a common ECD and marked as a harmful chemical (Wang et al., 2019). It also increases the bioavailability of other EDC such as BPA by competing for metabolic enzyme (Borman et al., 2017). In addition, the uterine cell types can response to estrogen and progesterone. Until now, the

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information about the possible effects of DEHP in uterus are very restricted. So, the possible effects of DEHP on uterine histology is followed.

DEHP AND UTERINE HISTOLOGY

Harmonized works between heterogeneous uterine cell types and maintained system homeostasis are critical in successful implantation and reproduction. The cell types are including luminal epithelial cells, glandular epithelial cells, stromal cells, smooth muscles, and uterine specific or unspecific immune cells. Harmonized works between cells are under the control of sex steroid hormones in system levels and these works are necessary to prepare the proper uterine environment for physiological responses (Cheon et al., 2002). Therefore, the uterine histology is typical for species by their reproduction patterns.

During radial patterning morphogenesis of uterus establishes the histological elements, endometrium, myometrium (middle muscular layer) which consists of a circularly arranged inner and longitudinally arranged outer smooth muscle layers, and perimetrium. The morphogenic processes during postnatal periods result in the organization and stratification of endometrial stroma, differentiation and growth of the myometrium, and coordinated development of the endometrial glands. In most eutherian mammals, endometrial and gland morphogenesis is a primarily postnatal event in the species-specific patterns by the placentation patterns and reproduction. It means that the functional layer of endometrium is established for the implantation and post differentiation (Walker, 2011; Spencer et al., 2012).

The complete maturation of the uterus is ultimately controlled by exposure to estrogen and progesterone. Estrogen and progesterone works through their specific receptors, ERs and PRs which mainly work as transcription factors in uterus (Okada et al., 2005). ER α is predominant in uterine tissue and expressed in all cell types. ER β is relatively lower expression levels in uterus and mainly located subepithelial stromal cells (Wang et al., 2000). PR is most strongly localized at epithelial cells during diestrus stage while at stroma during proestus stage (Ohta et al, 1993; Tan et al., 1999). ERs and PRs remain in inactive state by binding to an inhibitory protein complex containing heat-shock protein 90 (Hsp90) and activated by ligand binding (Nardulli & Shapiro, 1993; Klinge, 2001). Under the influence of estrogen, uterine wet weight and volume are increased (Shelby et al., 1996). Estrogen promotes proliferation and growth of endometrial cells and increases vascular permeability. Progesterone reduces ER levels and promotes cell differentiation and angiogenesis (Ma et al., 2001).

Among the uterine tissue, the endometrium undergoes huge changes during reproductive cycle. The endometrium is composed of surface epithelium, endometrial glands, and the lamina propria. The endometrial cells express ERs and PRs and are under the regulation of steroids. Luminal epithelial and glandular epithelia cells are simple cuboidal or columnar epithelial tissues (Li & Davis, 2007; Spencer et al., 2012). In rodent, during proestrus, luminal epithelial, stromal, myometrial cells proliferate and increase in number. The luminal and glandular epithelial cells are cuboidal shape. Apoptotic epithelial and stromal cells are observed (Marcus, 1974; Dharma & Nandedkar, 2001). During estrus, the luminal epithelial cells differentiate from cuboidal to columnar, with large cytoplasmic volume. Vacuolar degeneration and apoptosis can be observed in the luminal and glandular epithelium as the uterus reorganizes in preparation for implantation. Apoptotic luminal epithelial cell is observed (Dharma & Nandedkar, 2001). During metestrus, the epithelial cells are still large and columnar, but with greatly reduced cytoplasm. Glandular apoptosis is less frequent and fewer numbers of inflammatory cells are present in the lamina propria. Apoptotic luminal epithelial, glandular, and stromal cells are observed (Dharma & Nandedkar,

2001). During early diestrus, the glandular, stromal, and vascular cells are proliferating and leading to an increase in the thickness of the uterine endometrium. The luminal epithelial cells organize to single layer of tall columnar cell with basally located nuclei. Apoptotic stromal cell is observed (Dharma & Nandedkar, 2001).

Endometrial cell proliferation and differentiation are tightly regulated by estrogen and progesterone (Cheon et al., 2002; Singh et al., 2011). The adverse effects of high dose estrogen are well defined. Estrogen exposing during perinatal period induce cystic endometrial hyperplasia, squamous metaplasia, adenomyosis, and myometrial and general uterine hypoplasia (Houston et al., 2003). Besides, ER α overexpressors have higher number of apoptotic cells in the endometrial epithelium and reduced implantation sites (Tomic et al., 2007). Therefore, it is suspected that the estrogenic EDCs have similar effect on uterus. As suspected, DEHP can inhibit the binding of 17 β -estradiol to their receptors (Jobling et al., 1995) and induces estrogenic activity through ERs (Cavanagh et al., 2018). So far, it is known that the mode of action of DEHP is different between species. Tomonari et al. (2006), reported that DEHP for 65 weeks (0–100 mg/kg BW, from weaning (3 month of age) to sexual maturation (18 month) does not cause of abnormal histological changes in common marmosets. In the case of rat, 1,000 mg/kg/day exposing (gestation day 6–15) causes reducing the uterine weight (Hellwig et al., 1997; Ambe et al., 2019) but not by the exposing for 30 days (0–100 mg/kg BW/day) (Somasundaram & Manokaran, 2016). The uterine weight is increased by DEHP (133 μg/L drinking water) (Kim et al., 2018).

Uterine horns develop after birth to form an external myometrium surrounding the mesenchymal compartment (Cunha 1976; Kurita, 2011). In rat, the diameter of uterine horn is decreased by the treatment of DEHP (Somasundaram & Manokaran, 2016). In endometrial cells, MMP-2 and MMP-9 activity, cellular invasiveness, Erk phosporylation, and p21-activated kinase 4 expression are increased by the DEHP treatment (Kim et al., 2015). Development of the uterine glands is a particularly pivotal events to support the conceptus development in placental mammals (Burton et al., 2002; Gray et al., 2002). The interactions between epithelium and stroma in the gland forming area are critical and mediated by steroid hormones, paracrine factors, and the networks between 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 gland seems to be controversy. Somasundaram & Manokaran (2016) suggested that DEHP treatment decreases the number of endometrial glands and disrupts the glandular structure. However, some other groups reported opposite results. Richardson et al. (2018) showed an increase of the number of endometrial glands by DEHP (200 mg/kg/day) for 30 days. However, it is generally believed that DEHP has toxic effects on uterus as EDCs (Kim et al., 2018; Ambe et al., 2019). Chronic low-dose exposing of DEHP induce the histological changes. 50 μg/L DEHP in drinking water causes an increase in the number of glands (Kim et al., 2018).

Uterine mesenchyme directs and specifies the surfacing epithelial specification and epithelium is also required to support organization of endometrial stroma and myometrial differentiation (Cunha 1976, 1989; Kurita et al., 2001). Epithelial-mesenchymal interactions are mediated by intrinsic growth factor systems and the microenvironment by ECM (Spencer et al., 1993; Hu et al., 2004). DEHP exposing (200 μg/kg/day for 30 days) caused a reduction in epithelial cell proliferation in the uterus (Richardson et al., 2018). The thinning of the uterine layer is observed in 0–100 mg/kg BW/day for 30 day DEHP-treated rat (Somasundaram & Manokaran, 2016). However, chronic low-dose treatments of DEHP (133 and 1,330 μg/L drinking water) increases the endometrial thickness in mouse (Kim et al., 2018). On the other hand, DEHP can induce proliferation of the myometrial cells. A chronical 133 μg/L DEHP administration with drinking water increases the myometrial thickness (Kim et al., 2018).

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As described previously, the single compound phthalate can work as regulator of uterine histology. Besides, recent studies using phthalate mixture also showed similar effects on uterus. The exposing with environmentally relevant phthalate mixture (containing 35% DEP, 21% DEHP, 15% DBP, 15% DiNP, 8% DiBP, and 5% BBP) during gestation leads to changes in uterine histology in a multi-generational manner (Li et al., 2020). In Li et al. (2020) experiment, the phthalate mixture conflictingly does not effect on the uterine wet weight, endometrium size, number of glands, and inner or outer myometrium thickness in any generation. However, luminal epithelial cell proliferation is decreased in F1 generation. In 200 μg/kg/day phthalate mixture treated F1, 0.05% luminal epithelial cells were Ki67 positive. Interestingly, luminal epithelial cell proliferation is increased in F2 generation. The 200 μg/kg/day treatment caused 62.88% Ki67 positive of luminal epithelial cells. In all generation, a higher multilayered luminal epithelium is formed by the treatment of phthalate mixture. A phthalate mixture exposing during gestation cause a large, dilated endometrial glands in all generation. In some animals, gland invaded into the myometrial layer (Li et al., 2020).

So far, it is controversy whether DEHP can cause infertility of women. In animal model, high dose treatment of DEHP impaired female fertility (Schmidt et al., 2012). However, recently, my group evaluated the possible EDC working of low-dose DEHP in uterus by chronic exposing. However, the fertility of the parents and F1 offspring does not decreased (Cha et al., 2018; Kim et al., 2018). In women receiving IVF/ICSI treatments, the urinary levels of DEHP metabolites does not have correlation with clinical outcomes in the total population (Hauser et al., 2016; Deng et al., 2020).

In addition to the histological affects, DEHP can induce various cellular responses. In cellular level, DEHP causes of increases of the ER α and PR proteins in endometerium (Somasundaram & Manokaran, 2016). DEHP can induce oxidative stress in endometrial stroma cells (Cho et al., 2015). DEHP decreased the ER α protein expression level in Ishikawa human endometrial adenocarcinoma cells, and caused a weak decrease in VEGF secretion under hypoxia (Park et al., 2019). DEHP enhances proliferative activity and antiapoptotic effects in myometrial and leiomyoma cells (Kim et al., 2017). DEHP has agonistic effects to PR and induces higher GnRH receptor expression in the uterus and GnRH in hypothalamus in pubertal rats (Liu et al., 2016; Sheikh et al., 2016). DEHP induces the inflammation mediating peroxisome proliferator-activated receptor gamma (PPAR γ) of the primary cultured endometrial stroma cells without epithelial-mesenchymal transition (EMT) (Huang et al., 2016).

In uterus, estrogen and progesterone have opposing actions in uterine histological maintenance in a paracrine manner (Li et al., 2011; Chung et al., 2015). The complexity of steroid hormonal regulation and cellular responsibility in uterine function makes more complex to understand the possible effects of DEHP in uterine histological changes. However, the inhibitory works of EDCs can elucidate detrimental effects on health. Although further studies are needed more in model animals and human, in histological levels, DEHP cause various abnormal phenotypes. Those histological abnormal types and the change of the cellular responses may play a role in the pathogenesis of uterus.

CONCLUSION

Phthalates are widely used for help our daily life but some of the phthalates such as DEHP is toxic and hazardous to organ and ecosystem. Therefore, the usage of phthalates is under the control of government in many countries. Among the alternative materials of polymers, polyethylene, polypropylen, polyurethane, silicon and ethylene vinyl acetate are used. Development

of reproductive tracts in male and female is under the control of sex steroid hormone secreted from developing gonads. Therefore, the uterus is vulnerable to developmentally disruptive effects of ECDs in mammals.

Exposing to EDCs at critical period of differentiation for female reproductive tract can alter developmental programming and has profound effects on function disease outcome (Damgaard et al., 2002; Massé et al., 2009; Walker et al., 2011). In animal model, prenatal exposure to ECDs produce lesion in adult uteri such as altered steroid receptor concentration and responsiveness; persistent induction or de-regulation of gene expression; cystic endometrial hyperplasia, squamous metaplasia, myometrial hypoplasia, and general uterine hypoplasia (Spencer et al., 2012).

DEHP also has adverse effect in adult female uterine histological characters. It induces various cellular responses including the expression regulation of steroid hormone receptors and transcription factors. So far, it is controversy whether DEHP decreases the fertility or not. Recent research suggested that the chromic-low dose exposing of DEHP and environmentally relevant phthalate mixture may cause of histological modification in uterus and of disease in female such as endometriosis, hyperplasia, and myoma as suspected in high dose DEHP exposing.

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