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Review Article

Impact of DEHP (Di-2ethylhexyl-phthalate) Exposure on the Uterus in Rodent Models-An Overview

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Abstract

Female reproduction is affected by various factors such as stress, high-fat diet, and exposure to EDCs during a sensitive period like puberty, gestation, and lactation.

Introduction

Female reproduction is affected by various factors such as stress, high-fat diet, and exposure to EDCs during a sensitive period like puberty, gestation, and lactation [1-3]. The relationship between in utero and neonatal exposure to environmental toxicants is obvious as the offspring's impaired reproduction in adulthood as evidenced by epidemiological and animal studies. EDCs are one of the factors that affect the female reproductive system. It has been reported that EDCs target the hypothalamus, pituitary, ovary, and uterus [2].

An endocrine-disrupting compound was defined by the U.S. Environmental Protection Agency as "an exogenous agent that interferes 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". The endocrine disruptors are highly heterogenous and are classified as synthetic chemicals used as industrial solvents/ lubricants and their by-products Polychlorinated biphenyls (PCB), Polychlorinated biphenyls (PBBs), dioxins, Plastics (Phthalate), Pesticides [dichlorodiphenylchloroethane (DDT)], Fungicides (Vinclozolin) [4].

The endocrine glands release hormones into the bloodstream, these hormones regulate vital processes such as development and homeostasis. There are many factors that determine the availability of hormones such as gene and protein expression, exocytosis of vesicles containing peptide hormones, steroidogenesis of lipophilic hormones, transport via circulation. When a hormone within a physiologically relevant range is present, its action is executed in the target organ via receptors. The hormones (ligands) have a specific binding affinity with their respective receptors. Binding of ligand to its receptor activates membrane-bound signalling molecules which initiates a cascade of events ending up in specifically assigned functions. At environmentally relevant doses, few EDCs bind to hormone receptors and execute agonistic or antagonistic actions. The dose-response of EDCs is determined by hormone-receptor binding and availability. The conventional hormone concentration-response curve is a sigmoidal curve (Figure 1). In this case, minimal alterations in hormone concentration at the low end of the dose-response curve produce exponentially greater differences in effect, than similar changes in hormone concentration at the high end of the dose-response curve. So very low concentrations of EDCs could change the endogenous hormone concentration, producing an adverse effect. In addition, hormone receptors expressed at different concentrations will affect the various characteristics of the dose-response curve.

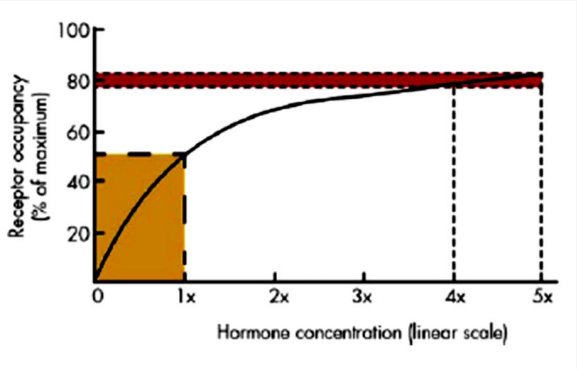

Figure 1 Schematic example of the relationship between receptor occupancy and hormone concentration. In this theoretical example, at low concentrations, an increase in hormone concentration from 0 to 1x causes an increase in receptor occupancy of approximately 50% (from 0 to 50%; see yellow box). Yet the same increment in hormone concentration at higher doses (from 4x to 5x) causes an increase in receptor occupancy of only approximately 4% (from 78 to 82%; see red box). (Reprinted from L. N. Vandenberg *et al.,* 2012)

Therefore, EDCs are capable of altering hormone receptors leading to fluctuations in the concentration of circulating hormones [5,6]. The impact of an EDC on the endocrine system may be permanent or temporary based on the duration of exposure and dosage.

Phthalates

Phthalates is one of the widely used endocrine-disrupting chemicals, used in a variety of consumer products like childrens' toys, food-packaging covers, medical tubing. This ubiquitous chemical can enter the human body and can disrupt hormonal functions from early life. The impact of early phthalate exposure will be reflected during late childhood and adolescence [7].

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Types of phthalates

Phthalates are classified into Di isononyl phthalate (DINP), Dibutylphthalate (DBP), Butyl benzyl phthalate (BBP), Diisododecyl phthalate (DNOP) [8,9], monocarboxy iso octyl phthalate, mono carboxy iso nonyl phthalate, Mono 3-carboxypropylphthalate, mono 2-ethyl 5-carboxy pentyl phthalate, monoethyl 5 hydroxyhexyl phthalate, mono-isobutyl phthalate, mono –n-butyl phthalate [10].

Mechanism of Phthalate Action

Peroxisome proliferator-activated receptors (PPARs), ligand-activated transcription factors that are members of the superfamily of steroid-thyroid-retinoid nuclear receptors, can be triggered by phthalates, which are endocrine disruptors [11]. The central nervous system, pituitary gland, testis, ovary, uterus, prostate, mammary gland, liver, and kidney all express PPAR isoforms (α, β or δ and γ) that are encoded by distinct genes [12-14]. In order to control the transcription of the target genes, activated PPARs form heterodimers with retinoid X receptors (RXR) and bind to PPAR response elements (PPRE) in the promoter [13]. Through EREs, PPAR/RXR and ER signaling pathways were found to interact in several studies [15,16]. Additionally, phthalates have been shown to stimulate ER-mediated estrogenic action, according to many investigations [17-22].

Impact of Phthalates on the Uterus

DEHP exposure to pregnant mice affected endometrial receptivity and reduced implantation sites via disrupting the MAPK signaling pathway and nuclear factor-B signaling pathways [23]. Uterotrophic assay explained that estradiol-induced uterine growth was hindered by dibenzyl phthalates [24]. In vitro study showed that exposure to DEHP and MEHP increased prostaglandin-F2α but decreased prostaglandin-E2 in bovine endometrial cells [25]. On the contrary, it has been shown that phthalate exposure does not have an impact on the uterine tissue. Chung et al. [26], reported that polypropylene and polyethylene terephthalate, extracted from plastic food containers did not increase uterine weight in Sprague-Dawley rats. Exposure to phthalate mixtures during the gestational period did not affect estradiol production except at high dose (500 mg/kg b.wt/day). However, a dose-dependent impact on the uterus was reported in F1, F2 and F3 offspring rats [27].

Phthalates and Endometriosis

A case-control study analyzed 35 cases and 24 controls, the cases exhibited higher concentrations of DEHP when compared with the controls [28]. A nonsignificant correlation between DEHP and endometriosis was reported in another case-control study which included 97 cases and 167 controls, whereas MEHP and endometriosis were having a significantly weak association in the same study [29]. Reddy et al. [30], analyzed the concentrations of DBP, BBP, DEHP, and DnOP in 49 women with endometriosis, 38 infertile controls, and 21 fertile controls, in which women with endometriosis had higher concentrations of DBP, BBP, DEHP, and DnOP ($p<0.01$), therefore higher concentrations of phthalates associated with the severity of endometriosis. The same study was repeated with a larger sample size of 85 cases and 135 controls which supported the previous study of Reddy et al. [31]. Wueve et al. [32], reported that in a cross-sectional study,1221 women took part, including 201 cases, MBP and endometriosis showed a nonsignificant association, but MEHP concentrations were higher in the control when compared to the cases. An epidemiological study conducted by Huang et al. [33], showed that the metabolites of the phthalate (MBP and MEHP) were nonsignificantly increased in endometriosis cases (n=28) compared with the controls. Banu et al., (2023), reviewed the roles of phthalate in the pathogenesis of endometriosis will help [34].

Impact of Phthalates on Puberty

Studies on phthalate exposure and puberty in humans resulted in mixed outcomes. A review by Jurewicz and Hanke et al. [35], reported a link between urinary levels of phthalates and pubertal gynecomastia, as well as a positive correlation between serum levels of phthalates and premature thelarche and precocious puberty in girls. In another case-control study conducted in Taiwan girls showed that urinary phthalate metabolites were significantly higher in girls with precocious puberty [36]. Higher levels of kisspeptin were recorded in girls with precocious puberty suggesting that phthalates might act by increasing kisspeptin activity, which in turn promotes puberty [37]. Urinary concentrations of high molecular weight phthalates (including DEHP), were linked with delayed pubic hair acquisition, and age at breast development was older fifth quintile of urinary MBzP concentrations compared to those in the first quintile in a study including 1200 peripubertal girls [38]. Contradictorily in a small cohort of Puerto Rican girls, significantly higher levels of high molecular weight phthalates were identified in 68% of blood samples from precocious thelarche patients [39]. Few experimental studies reported that DBP and butyl benzyl phthalate (BBP) exposure did not affect vaginal opening in rats [40], whereas other experiment states that DBP exposure induced earlier pubertal timing in female Sprague-Dawley rats [41]. A dose-dependent effect of DBP on vaginal opening and completely hindered vaginal opening at high doses (750 and 1000 mg/kg/d) in were observed in phthalate-exposed rats [42]. Late gestational to lactational exposure to DBP in rats resulted in poor mammary alveolar branching and hypoplasia in the adult female offspring [43]. In contrast, a marginal acceleration of mammary gland growth by increasing the proliferative index of TEBs and delayed pubertal onset was observed in in utero exposure to BBP [44,45]. Several animal studies reported adverse female reproductive and developmental outcomes where the exposure of specific phthalates [diethyl phthalate (DEP), di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DnBP), and diisobutyl phthalate (DiBP)] significantly reduced embryo survival, increased the incidence of resorptions and abortion rate, decreased the number and size of litters in rats [47,48]. Zhou et al., (2017) reported that prenatal exposure to a phthalate mixture at 200 mg/kg/day increased the time to get pregnant in F1 mice (3 months of age) although they exhibited normal mating index [49].

DEHP

DEHP are yellow, odourless oily liquids which have a slight solubility in water and used to impart flexibility to the Polyvinylchloride materials. The high hydrophobicity leads to strong sorption of the high molecular weight phthalates to organic matter [50]. The bioconcentration factor of DEHP is high, BCF-115 to 851 whereas the water solubility is low (Versar, Syracuse Research Corporation). With increasing alkyl chain length, the primary biodegradation half-life increases [50]. DEHP has a high tolerated daily intake whereas for phthalates there is Temporary Tolerated daily Intake [51]. (Figure 2 and Table 1).

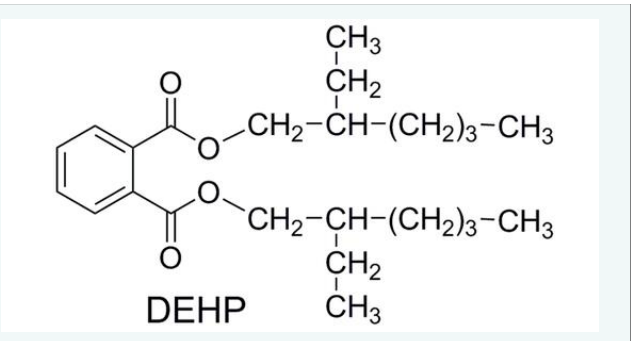

Figure 2 Structure of DEHP (Smerieri *et al.,* 2015).

Table 1: PHYSICO-CHEMICAL PROPERTIES of DEHP (Australia's Di-(-2-Ethylhexyl) phthalate emission report -2020 to 2021)

CHEMICAL FORMULA	$C_{24}H_{38}O_4$
MOLECULAR WEIGHT	390.62
VAPOUR PRESSURE	1.32 mmHg at 200°C
MELTING POINT	-50°C
BOILING POINT	230°C at 5 mm Hg
SPECIFIC GRAVITY	0.986 at 20/20°C

METABOLISM OF DEHP

DEHP is made up of dialkyl or alkyl/aryl esters of 1,2-benzenedicarboxylic acid [52]. It is a high molecular weight compound that is first hydrolysed by pancreatic lipases, liver estrases, and nonspecific esterases in the blood [53,54]. The longer chain length dialkyl phthalates are DEHP, DnOP, and DiNP are hydrolyzed to their respective monoester phthalates which are again extensively metabolized by oxidation of their alkyl side chain. Specifically, DEHP is reduced to its primary monoester metabolite, mono (2-ethylhexyl)phthalate (MEHP), which extends as a multistep oxidative pathway by N- and N-1-oxidation of the aliphatic side chain, is converted to mono (2-ethyl-5-hydroxyhexyl) phthalate (5OHMEHP) and mono (2-ethyl-5-oxo-hexyl) phthalate (5oxo-MEHP), and to mono (2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) and mono 2-(carboxymethyl) hexylphthalate (2cx-MMHP) and it is removed after conjugation with glucuronide.

ABSORPTION

In rats, phthalates are quickly absorbed from intestine in a concentration-dependent range, especially in the form of monoesters, hydrolysed by gut lipases. Kluwe et al. [55], reported that more than 90% of DBP and 40–50% of DEHP added to the feed were detected in urine following oral administration to rodents, indicating that phthalates present in the food were absorbed well. The oral absorption rate for DEHP was estimated in a healthy Caucasian male volunteer by measuring the levels of metabolites in urine [56]. Sixty seven percent (67%) of the dose was excreted in urine after 24 h, followed by an extra 3.8% on the second day, it showed that the major part of the ingested DEHP is systemically absorbed and excreted in urine.

DISTRIBUTION

Phthalates and their metabolites are widespread throughout the body in all tissues. Several reports state that DEHP distribution in different species exhibits highest concentrations in liver and kidneys. Phthalates were also detected in amniotic fluid, breast milk, seminal fluid, saliva, and placenta in human beings [57-60].

BIOTRANSFORMATION

After oral ingestion, nonspecific esterases and lipases cleave di-ester phthalates into their respective monoester metabolites in the GIT. Absorption is completed followed by the conversion of the monoesters are to secondary metabolites by many oxidation and hydroxylation reactions, which are then eliminated through urine or combined to glucuronic acid before excretion. Approximately 80–90% of the urinary metabolites is conjugated to glucuronic acid in adult humans [61]. The first step of biotransformation has high interspecies variability evidenced by kinetic studies in different species, it indicates that lipase activity may have a significant variability between species [62].

Impact of DEHP exposure on the female reproductive system

Experimental and observational studies, documented biomonitoring reports states the DEHP has adverse effects on the female reproductive system by modulating the ovarian and uterine functions. DEHP can pass through the placenta and breast milk, if exposed during gestation, it affects the foetus and germ cells thereby affecting the reproduction of the progeny in adulthood.

Impact of DEHP exposure on Vaginal Opening

Grande et al. [63], reported that oral doses of DEHP at 5,15,45,135,405 mg/kg/d from gestation day 6 to lactation day 21 led to a decrease in the day of vaginal opening at all doses and delay in the first estrous at 135 and 405 mg/kg/d. Rattan et al. [64], reported that in F1 generation mice 200µg/kg accelerated vaginal opening, in F2 generation mice 500 mg/kg DEHP exposure accelerated the vaginal opening, in F3 generation mice 200 µg/kg, 500 mg/kg, 750 mg/kg DEHP exposure accelerated the vaginal opening with no change in the levels of serum estradiol at PND 21. DEHP at 30 or 300 mg/kg by gavage from GD 8 to PND 21 delayed vaginal opening in the F1 offspring rats [65].

Impact of DEHP exposure on the length of estrous cycle

Few studies have shown that DEHP exposure increased estrous cycle length and induced irregular estrous cycles after exposure to 25 mg/km3 through inhalation, or oral doses greater than 1000 mg/kg/d in Wistar and Sprague–Dawley rats, and in mice [66-68]. Oral administration of 3000mg/kg/d DEHP for Sprague Dawley rats starting from 2 weeks before mating and till day 7 of pregnancy for a female fertility study and to another set 3000 mg/kg/d DEHP was administered for 4 weeks. Irregular estrous cycle was recorded in the 2-4week study group and decrease in the body weight and atropy in the uterus [69]. Estrous cycle was altered in the F1 generation at the maternal exposure of 200 µg/kg/day & 500 mg/kg/day, increased the time spent in estrus in 20 µg/kg/day and 200 µg/kg/day in F2 generation and in 20 µg/kg/day F3 generation rats in the experiment by Rattan et al., [64].

Impact of DEHP Exposure on Reproductive Indices

Many reports stated using high doses of DEHP found negative impact on pregnancy. Maternal weight and food consumption, number of pups born, pup weight and the rate of post-implantation loss were affected at oral doses from 500 to 1500 mg/kg/d as well as 10 ml/kg/d and 1% in diet in mice and rats [70-77]. Dietary administration of 500 mg/kg/d DEHP for 8 weeks to CH3/N mice led to degeneration in the blastocysts in the F1 pre-implantation embryos [78]. Rattan et al. [64], reported that fertility index was reduced in the F1 generation at 20µg/kg/day and 200µg/kg/day after the prenatal DEHP exposure. The pregnant mice received DEHP at 0, 250, 500 and 1000 mg/kg/day from day 1 of gestation to day 6 of gestation and it was found that the number of implantation sites was significantly reduced in the 1000 mg/kg/day DEHP group [79].

Impact of DEHP Exposure on Adult Uterus

The adult female Wistar rats were treated with DEHP orally with 1,10,100 mg/kg/body weight/day and it led to increase in ovarian hormones and their receptor levels in the uterus and morphological abnormalities such as thinning of the layers and disruption of glandular epithelium [80]. Kim et al., 2018 [81] stated that ten- to twelve-week-old CD-1 mice were fed drinking water containing NP (50 or 500µg/L) or DEHP containing (133 or 1330µg/L) for 10 weeks and it resulted in endometrial and myometrial thickness increased in 133 and 1330µg/L DEHP, 50 & 500 µg/L NP. In addition to it, the height of luminal epithelial cell decreased in NP groups and increased in 50µg/L DEHP group.

Impact of DEHP exposure on the ovary and uterus of F1 progeny

Zhang et al. [82], stated that water containing DEHP 40µg /kg/ day or normal saline was gavaged to CD-1 mice from 0.5 dpc to 18.5 dpc, it led to reduction in maternal serum estradiol levels and induced ovarian development deficiency in the F1 offspring mice. Somasundaram et al.

[83], reported that 1,10,100mg /kg/DEHP, gestational and lactational exposure to Wistar rats led to decreased body weight, ovarian weight, uterine weight, and serum estradiol levels in F1 offspring rats, in addition to it the expression of proteins involved in steroidogenesis were altered and the effect was profound at 100mg/kg/day DEHP exposure.

Impact of DEHP exposure on the miRNAs

miRNAs

miRNAs are short sequences, made up of 22 nucleotides, non-coding RNAs. miRNAs regulate post-transcriptional protein expression. miRNAs are encoded by a single gene controlled by their cognate promoters and regulatory sequences and are arranged in clusters [84]. They are transcribed from intergenic sequences of the protein-coding genes using RNA polymerase II and RNA polymerase III [85,86]. Drosha RNase III endonuclease or by an alternative Drosha-independent mirtron cleaves the primary miRNAs into stem-loop pre-miRNAs which are 60-70 nt long. The pre-miRNAs are then transported to the cytoplasm and it is further cleaved by the Dicer enzyme, in this process the loop structure is removed and mature miRNA duplexes are formed overhanging at the 3' end. miRNA duplexes are guided to the Argonaute (AGO) protein part of the RNA-induced silencing complex (RISC) by the Dicer and the AGO protein uncoils the duplexes, resulting in single-stranded miRNA-5p and miRNA-3p products. The mature miRNA is incorporated into RISC. The miRNA-RISC complex binds to the target mRNAs and represses the ribosomal assembly and deactivates the target mRNA. The mRNAs which are bound to the miRNA-RISC complex are further stored in P-bodies, and cytoplasmic structures, and either released upon a cellular signal or destroyed [87,88]. The physiopathological profile of the cell, the microenvironment, and the milieu regulates the biogenesis of miRNAs and the expression of the miRNAs in the endometrium is cell-dependent. Single nucleotide polymorphism and epigenetic modifications, interactions with RNA-binding proteins regulate miRNA expression and maturation [88]. MiRNAs are distinctive based on their complementarity to their target miRNA [84]. The extent of complementarity determines whether the target mRNA is degraded or its translation is blocked. MiRNAs act according to the target mRNA binding site and can activate gene expression [89]. A single miRNA can regulate many proteins and one mRNA can also be regulated by various miRNAs [88]. (Figure 3).

Association of DEHP exposure and the miRNA levels

Chang et al. [90], stated the role of MEHP-induced reactive oxygen species (ROS) for genotoxicity and the toxicity of MEHP downregulated the miR-let-7a and miR-125b in AS52-mutant cell (ASMC) clones. The carcinogenicity of MEHP in Chinese hamster AA8, UV5, and EM9 ovary cells, and it's capacity to induce epigenetic modifications were proved (Chang et al., 2017). Forty maternal urine samples were analyzed for MEHP, MBzP, MBP, MiBP, and total BPA were analysed. The Limits of detection (LOD) for metabolites were fixed at 6.41, 0.23, 1.08, 0.15 and 22.2 ng/mL for BPA, MiBP, MBP, MBzP, and MEHP respectively. For concentrations below the LOD, a value equal to each sample's specific LOD divided by the square root of 2 was used. Further selected miRNAs were analyzed in the serum of 22 GDM patients and 18 non-diabetic women. It was found that relative expression levels of miR-29a-5p, miR-29a-3p and miR-330-3p were significantly up-regulated in the serum from GDM patients compared to serum from the non-diabetic women [91]. Bai et al. [92], reported that ten urinary phthalate metabolites including mono-n-butyl phthalate (MBP), mono-iso-butyl phthalate (MiBP), mono-benzyl phthalate (MBzP), mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-n-octyl phthalate (MOP), mono-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5- hydroxyhexyl) phthalate (MEHHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), were determined. The limits of detection (LODs) for urinary phthalate metabolites were fixed in the range of 0.2–2.0 ng/mL, and values less than the LODs were imputed with LOD/$\sqrt{2}$. It was observed that the spike recoveries of the 10 phthalate metabolites ranged from 61.0% to 91.3% and positive associations of phthalate metabolites mixture with miR-146a, miR-125b, and miR-222 [92].

Conclusion

The available research mainly focuses on the impact of maternal exposure to DEHP on the ovaries of offspring animals. However, the uterus is the organ targeted by ovarian steroids, and the effect of ancestral DEHP exposure on the uterus of offspring has not been studied. DEHP is a weak estrogenic compound and can disrupt the actions of estrogen in the uterus, leading to impairment of uterine development and function. Prenatal and postnatal DEHP exposure affects the uterine environment, increasing the risk of miscarriage and pre-term delivery of the fetus.

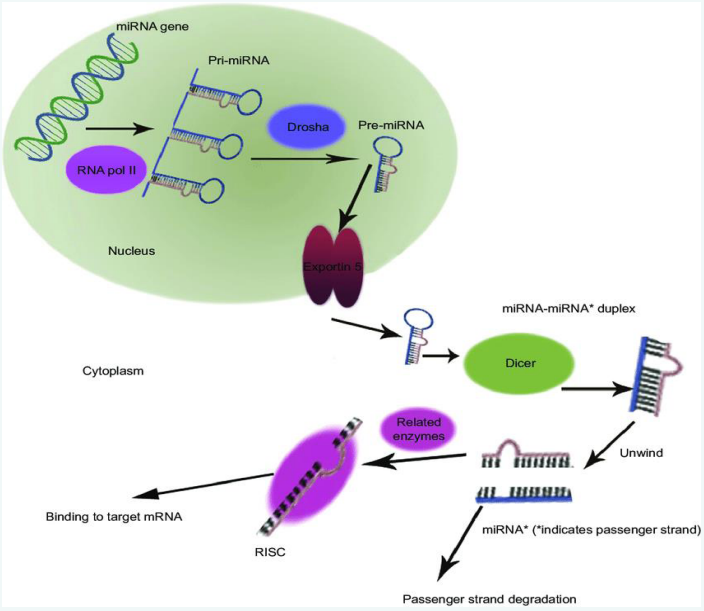

Figure 3 miRNA Biogenesis (Luan *et al.,* 2015)

Additionally, there is a possible transfer of uterine dysfunction to the offspring through miRNAs, which are an epigenetic signature passed on through the maternal lineage, further exacerbated in adulthood. It is essential to study how phthalates alter the epigenetic assets of cells, as it can help us understand the uterine dysfunction caused by EDCs.

Method of search

Keywords such as Phthalate, DEHP, female, Rat, Ovary, Uterus, and miRNA were used during Pubmed and Google searches. Research and review articles which had relevance to the topic were chosen to draft the review.

References

1. Schenker JG, Meirow D, Schenker E. Stress and human reproduction. European journal of obstetrics gynecology and reproductive biology. 1992; 45: 1–8.
2. Zama AM, Uzumcu M. Epigenetic effects of endocrine-disrupting chemicals on female reproduction: an ovarian perspective. Front Neuroendocrinol. 2010; 31: 420–439.
3. Natalie M Hohos, Malgorzata E Skaznik-Wikiel. High-Fat Diet and Female Fertility. Endocrinology. 2017; 158: 2407–2419.
4. Diamanti Kandarakis E, Bourguignon JP, Giudice LC. Endocrine-disrupting chemicals: an Endocrine SocietyScientific Statement. Endocr Rev. 2009; 30: 293–342.
5. Xu XB, He Y, Song C, Ke X, Fan SJ, Peng WJ, et al. Bisphenol A regulates the estrogen receptor alpha signaling in developing hippocampus of male rats through estrogen receptor. Hippocampus. 2014; 24: 1570-80.
6. Martinez Arguelles DB, Campioli E, Lienhart C. In utero exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate induces long-term changes in gene expression in the adult male adrenal gland. Endocrinology. 2014; 155: 1667–1678.
7. Sears CG, Braun JM. Phthalate Exposure, Adolescent Health, and the Need for Primary Prevention. Endocrinol Metab Clin North Am. 2020; 49: 759–770.
8. Valles EG, Laughter A, Dunn CS, Cannelle S, Swanson CL, Cattley RC, et al. Role of the peroxisome proliferator-activated receptor α in responses to diisononyl phthalate. Toxicology. 2003; 191: 211-225.
9. Seo KW, KB Kim, YJ Kim, JY Choi, KT Lee, KS Choi. Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats. Food Chem Toxicol. 2004; 42: 107-114.
10. Lych JL, Gutleb AC, Bergman A, Eriksen GS, Murk AJ, Ropstad E, et al. Reproductive and developmental toxicity of phthalates. J Toxicol Environ Health B Crit Rev. 2009; 12: 225–249.
11. Latini G, Scoditti E, Verrotti A, De Felice C, Massaro M. Peroxisome proliferator-activated receptors as mediators of phthalate-induced effects in the male and female reproductive tract: epidemiological and experimental evidence. PPAR Res. 2008; 359267.
12. Braissant O, Foufelle F, Scotto C, Dauça M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology. 1996; 137: 354–366.
13. Lemberger T, Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol. 1996; 12: 335–363.
14. Froment P, Gizard F, Defever D, Staels B, Dupont J, Monget P. Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. J Endocrinol. 2006; 189: 199–209.
15. Keller H, Givel F, Perroud M, Wahli W. Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. Mol Endocrinol. 1995; 9: 794–804.
16. Corton PJ. Lapinskas. Peroxisome proliferator-activated receptors: mediators of phthalate ester-induced effects in the male reproductive tract? Toxicol Sci. 2005; 83: 4–17.
17. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Respect. 1995; 103: 582-587.
18. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ health perspect. 1995; 103: 113–122.
19. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ health perspect. 1997; 105: 802–811.
20. Andersen HR, Andersson AM, Arnold SF, Autrup H, Barfoed M, Beresford NA, et al. Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals. Environ health perspect. 1999; 107: 89–108.
21. TR Zacharewski, MD Meek, JH Clemons, ZF Wu, MR Fielden, JB Matthews. Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. Toxicol Sci. 1998; 46: 282–293.
22. Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T, Kojima H. Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. Toxicology. 2005; 210: 223–233.
23. Toft G, Jönsson BA, Lindh CH, Jensen TK, Hjollund NH, Vested A, et al. Association between pregnancy loss and urinary phthalate levels around the time of conception. Environ health perspect. 2012; 120: 458–463.
24. Zhang Z, Hu Y, Zhao L. Estrogen agonist/antagonist properties of dibenzyl phthalate (DBzP) based on in vitro and in vivo assays. Toxicol Lett. 2011; 207: 7–11.
25. Wang X, Shang L, Wang J, Wu N, Wang S. Effect of phthalate esters on the secretion of prostaglandins (F2αand E2) and oxytocin in cultured bovine ovarian and endometrial cells. Domest Anim Endocrinol. 2010; 39: 131–136.
26. Chung BY, Kyung M, Lim SK. Uterotrophic and Hershberger assays for endocrine disruption properties of plastic food contact materials polypropylene (PP) and polyethylene terephthalate (PET). J Toxicol Environ Health A. 2013; 76: 624–634.
27. Li K, Liszka M, Zhou C, Brehm E, Flaws JA, Nowak RA. Prenatal exposure to a phthalate mixture leads to multigenerational and transgenerational effects on uterine morphology and function in mice. Reprod Toxicol. 2020; 93: 178–190.
28. Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F, et al. High plasma concentrations of di-(2-ethylhexyl)phthalate in women with endometriosis. Hum Reprod. 2003; 18: 1512–1515.
29. SH Kim, S Chun, JY Jang, HD Chae, CH Kim, BM Kang. Increased plasma levels of phthalate esters in women with advanced-stage endometriosis: a prospective case-control study. Fertil Steril. 2011; 95: 357–359.
30. Reddy BS, Rozati R, Reddy BV, Raman NV. Association of phthalate esters with endometriosis in Indian women. BJOG. 2006; 113: 515–520.

31. Reddy BS, Rozati R, Reddy S, Kodampur S, Reddy P, Reddy R. High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study. Fertil steril. 2006; 85: 775–779.

32. Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA. Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999-2004. Environ health perspect. 2010; 118: 825–832.

33. Huang PC, Tsai EM, Li WF. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. Hum Reprod. 2010; 25: 986-994.

34. Dutta S, Banu SK, Arosh JA. Endocrine disruptors and endometriosis. Reprod toxicol. 2023; 115: 56–73.

35. Jurewicz J, Hanke W. Exposure to phthalates: reproductive outcome and children health. A review of epidemiological studies. Int J Occup Med Environ Health. 2011; 24: 115–141.

36. Janesick A, Blumberg B. Obesogens, stem cells and the developmental programming of obesity. Int J Androl. 2012; 35: 437–448.

37. Chen CY, Chou YY, Wu YM, Lin CC, Lin SJ, Lee CC. Phthalates may promote female puberty by increasing kisspeptin activity. Hum Reprod. 2013; 28: 2765–2773.

38. Wolff MS, Teitelbaum SL, McGovern K. Phthalate exposure and pubertal development in a longitudinal study of US girls. Hum Reprod. 2014; 29: 1558–1566.

39. Colón I, Caro D, Bourdony CJ, Rosario O. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ health perspect. 2000; 108: 895–900.

40. Ahmad R, Verma Y, Gautam AK, Kumar S. Assessment of estrogenic potential of di-n-butyl phthalate and butyl benzyl phthalate in vivo. Toxicol Ind Health. 2015; 31: 1296-1303.

41. Hu J, Du G, Zhang W. Short-term neonatal/prepubertal exposure of dibutyl phthalate (DBP) advanced pubertal timing and affected hypothalamic kisspeptin/GPR54 expression differently in female rats. Toxicology. 2013; 314: 65–75.

42. Ding Y, Gao Y, Shi R, Zhou YJ, Tian Y. Effects of in utero exposure to di(2-ethylhexyl) phthalate on sexual development in female offspring. Zhonghua Yu Fang Yi Xue Za Zhi. 2010; 44: 150–153.

43. Lee KY, Shibutani M, Takagi H. Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology. 2004; 203: 221–238.

44. Moral R, Santucci-Pereira J, Wang R, Russo IH, Lamartiniere CA, Russo J. In utero exposure to butyl benzyl phthalate induces modifications in the morphology and the gene expression profile of the mammary gland: an experimental study in rats. Environ Health. 2011; 10: 5.

45. Moral R, Wang R, Russo IH, Mailo DA, Lamartiniere CA, Russo J. The plasticizer butyl benzyl phthalate induces genomic changes in rat mammary gland after neonatal/prepubertal exposure. BMC Genomics. 2007; 8: 453.

46. Saillenfait AM, Sabate JP, Gallissot F. Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-nbutyl phthalate, administered by gavage to rats. Toxicol Lett. 2006; 165: 39–46

47. Schmidt JS, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Effects of di (2-ethylhexyl) phthalate (DEHP) on female fertility and adipogenesis in C3H/N Mice. Environ. Health Perspect. 2012; 120: 1123–1129.

48. Zhou C, Gao L, Flaws JA. Prenatal exposure to an environmentally relevant phthalate mixture disrupts reproduction in F1 female mice. Toxicol Appl Pharmacol. 2017; 318: 49–57.

49. Cousins I, Clark K, Mackay D. Assessment of critical exposure pathways. In The Handbook of Environmental Chemistry: Phthalate Esters. Staples CA, ed. Berlin, Germany: Springer Verlag. 2003; 3: 227-262.

50. Hines CJ, Hopf NB, Deddens JA, Silva MJ, Calafat AM. Estimated daily intake of phthalates in occupationally exposed groups. J Expo Sci Environ Epidemiol. 2011; 21: 133-141.

51. Latini G. Monitoring phthalate exposure in humans. Clin Chim Acta. 2005; 361: 20–29.

52. Calafat AM, Needham LL. What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data? Environ health perspect. 2009; 117: 1481–1485.

53. Rusyn I, Peters JM, Cunningham ML. Modes of action and species-specific effects of di-(2-ethylhexyl) phthalate in the liver. Crit rev toxicol. 2006; 36: 459–479.

54. Kluwe WM, Huff JE, Matthews HB, Irwin R, Haseman JK. Comparative chronic toxicities and carcinogenic potentials of 2-ethylhexyl-containing compounds in rats and mice. Carcinogenesis. 1985; 6: 1577–1583.

55. Koch HM, Preuss R, Angerer J. Di (2-ethylhexyl) phthalate (DEHP): human metabolism and internal exposure-- an update and latest results. Int J Androl. 2006; 29: 155–185.

56. Koch HM, Rossbach B, Drexler H, Angerer J. Internal exposure of the general population to DEHP and other phthalates—determination of secondary and primary phthalate monoester metabolites in urine. Environ Res. 2003; 93: 177–185.

57. Hauser Russ, Susan Duty, Linda Godfrey-Bailey, Antonia M Calafat. Medications as a source of human exposure to phthalates. Environmental Health Perspectives. 2004; 112: 751-753.

58. Silva MJ, Barr DB, Reidy JA. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES). Environ Health Perspect. 2004; 112: 331–338.

59. Mortensen GK, Main KM, Andersson AM, Leffers H, Skakkebaek NE. Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS). Anal Bioanal Chem. 2005; 382: 1084-92.

60. Peck CC, Albro PW. Toxic potential of the plasticizer Di(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. Environ Health Perspect. 1982; 45: 11-17.

61. Ito Y, Yokota H, Wang R, Yamanoshita O, Ichihara G, Wang H, et al. Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets. Arch toxicol. 2005; 79: 147–154.

62. Grande SW, Andrade AJM, Talsness CE, Grote K, Chahoud I. A dose-response study following in utero and lactational exposure to Di(2-ethylhexyl)phthalate:Effects on female rat reproductive development. Toxicol Sci. 2006; 91: 247–254.

63. Rattan S, Brehm E, Gao L, Flaws JA. Di(2-ethylhexyl) phthalate exposure during prenatal development causes adverse transgenerational effects on female fertility in mice. Toxicol Sci. 2018; 163: 420–429.

64. Nardelli TC, Albert O, Lalancette C, Culty M, Hales BF, Robaire B. In Utero and Lactational Exposure Study in Rats to Identify Replacements for Di(2-ethylhexyl) Phthalate. Scic rep. 2017; 7: 3862.

65. Hirosawa N, Yano K, Suzuki Y, Sakamoto Y. Endocrine disrupting effect of di-(2-ethylhexyl) phthalate on female rats and proteome analyses of their pituitaries. Proteomics. 2006; 6: 958–971.

66. Ma M, Kondo T, Ban S, Umemura T, Kurahashi N, Takeda M, et al. Exposure of prepubertal female rats to inhaled di(2-ethylhexyl) phthalate affects the onset of puberty and postpubertal reproductive functions. Toxicol sci. 2006; 93: 164–171.

67. Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). Reprod Toxicol. 2012; 34: 43–50.

68. Takai R, Hayashi S, Kiyokawa J, Iwata Y, Matsuo S, Suzuki M, et al. Collaborative work on evaluation of ovarian toxicity. 10) Two- or four-week repeated dose studies and fertility study of di-(2-ethylhexyl) phthalate (DEHP) in female rats. J toxicol sci. 2009; 34: 111–119.

69. Tomita I, Nakamura Y, Yagi Y, Tutikawa K. Teratogenicity/ fetotoxicity of DEHP in mice. Environ Health Perspect. 1982; 45: 71–5.

70. Tyl RW, Price CJ, Marr MC, Kimmel CA. Developmental toxicity evaluation of dietary di(2-ethylhexyl) phthalate in Fischer 344 rats and CD-1 mice. Fundam Appl Toxicol. 1988; 10: 395–412.

71. Anonymous. Reproductive toxicology: diethylhexyl phthalate, juvenile exposure, mice. Environ Health Perspect. 1997; 105: 243–244.

72. Peters JM, Taubeneck MW, Keen CL, Gonzalez FJ. Di(2-ethylhexyl) phthalate induces a functional zinc deficiency during pregnancy and teratogenesis that is independent of peroxisome proliferator-activated receptor-alpha. Teratology. 1997; 56: 311–16.

73. Moore RW, Rudy TA, Lin TM. Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. Environ Health Perspect. 2001; 109: 229–37.

74. Jarfelt K, Dalgaard M, Hass U. Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. Reprod Toxicol. 2005; 19: 505–15.

75. Dalsenter PR, Santana GM, Grande SW. Phthalate affect the reproductive function and sexual behavior of male Wistar rats. Hum Exp Toxicol. 2006; 25: 297–303.

76. Pocar P, Fiandanese N, Secchi C, Berrini A, Fischer B, Schmidt JS, et al. Exposure to di(2-ethyl-hexyl) phthalate (DEHP) in Utero and during lactation causes long-term pituitary-gonadal axis disruption in male and female mouse offspring. Endocrinology. 2012; 153: 937–948.

77. Schmidt JS, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Effects of di (2-ethylhexyl) phthalate (DEHP) on female fertility and adipogenesis in C3H/N Mice. Environ. Health Perspect. 2012; 120: 1123–1129.

78. Li R, Yu C, Gao R, Liu X, Lu J, Zhao L, et al. Effects of DEHP on endometrial receptivity and embryo implantation in pregnant mice. J hazard mater. 2012; 241-242: 231–240.

79. Somasundaram DB, Manokaran K, Selvanesan BC, Bhaskaran RS. Impact of di-(2-ethylhexyl) phthalate on the uterus of adult Wistar rats. Hum Exp Toxicol. 2017; 36: 565–572.

80. Kim J, Cha S, Lee MY, Hwang YJ, Yang E, Ryou C, et al. Chronic low-dose nonylphenol or di-(2-ethylhexyl) phthalate has a different estrogen-like response in mouse uterus. Dev Reprod. 2018; 22: 379-391.

81. Zhang XF, Zhang T, Han Z, Liu JC, Liu YP, Ma JY, et al. Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reprod fertile dev. 2015; 27: 1213–1221.

82. Somasundaram DB, Selvanesan BC, Ramachandran I, Bhaskaran RS. Lactational Exposure to di (2-ethylhexyl) Phthalate Impairs the Ovarian and Uterine Function of Adult Offspring Rat. Reprod Sci. 2016; 23: 549–559.

83. Ambros V. The functions of animal microRNAs. Nature. 2004; 431: 350–355.

84. Lee KY, Shibutani M, Takagi H. Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology. 2004; 203: 221–238.

85. Borchert G, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol. 2006; 13: 1097–1101.

86. Klinge CM. miRNAs and estrogen action. Trends Endocrinol Metab. 2012; 23: 223-233.

87. De Sousa MC, Gjorgjieva M, Dolicka D, Sobolewski C, Foti M. Deciphering miRNAs' Action through miRNA Editing. Int J Mol Sci. 2019; 20: 11.

88. Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature. 2008; 455: 58–63.

89. Chang YJ, Tseng CY, Lin PY, Chuang YC, Chao MW. Acute exposure to DEHP metabolite, MEHP cause genotoxicity, mutagenesis and carcinogenicity in mammalian Chinese hamster ovary cells. Carcinogenesis. 2017; 38: 336–345.

90. Martínez-Ibarra A, Martínez-Razo LD, Vázquez-Martínez ER, Martínez-Cruz N, Flores-Ramírez R, García-Gómez E, et al. Unhealthy Levels of Phthalates and Bisphenol A in Mexican Pregnant Women with Gestational Diabetes and Its Association to Altered Expression of miRNAs Involved with Metabolic Disease. Int J Mol Sci. 2019; 20: 3343.

91. Bai C, Yang H, Zhao L, Liu L, Guo W, Yu J, et al. The mediating role of plasma microRNAs in the association of phthalates exposure with arterial stiffness: A panel study. Environ res. 2022; 212: 113469.