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Review

Reproductive toxic potential of phthalate compounds – State of art review

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ABSTRACT

Phthalates are pervasive compounds, and due to the ubiquitous usage of phthalates, humans or even children are widely exposed to them. Since phthalates are not chemically bound to the plastic matrix, they can easily leach out to contaminate the peripheral environment. Various animal and human studies have raised vital health concern including developmental and reproductive toxicity of phthalate exposure. The present review is based upon the available literature on phthalates with respect to their reproductive toxic potential. Common reproductive effects such as declined fertility, reduced testis weight, variations in accessory sex organs and several female reproductive disorders appeared to be largely associated with the transitional phthalates. Among the higher molecular weight phthalates (≥ C7), di-isononyl phthalate (DINP) produces some minor effects on development of male reproductive tract and among low molecular weight phthalates (≤C3), di-methyl (DMP) and di-isobutyl (DIBP) phthalate produce some adverse effects on male reproductive system. Whereas transitional phthalates such as di-butyl phthalate, benzyl butyl phthalate, and di-(2-ethylhexyl) phthalate have shown adverse effects on female reproductive system. Owing to these, non-toxic alternatives to phthalates may be developed and use of phthalates could be rationalized as an important issue where human reproduction system is involved. Though, more epidemiological studies are needed to substantiate the reported findings on phthalates.

Abbreviations: 17α-OHase, 17α-hydroxylase; AF, amniotic fluid; AGD, anogenital distance; AR, androgen receptor; BBP, butyl benzyl phthalate; CAP, cellulose acetate phthalate; CPP, central precocious puberty; DAP, di-alkyl phthalate; DBP, dibutyl phthalate; DEHP, di-2-ethylhexyl phthalate; DEP, diethyl phthalate; DIBP, di-isobutyl phthalate; DIDP, di-isodocyl phthalate; DIHP, di-isoheptyl phthalate; DINP, di-isononyl phthalate; DMEP, di-methylethyl phthalate; DMP, dimethyl phthalate; DNP, di-nonyl phthalate; DPP, di-pentyl phthalate; Eds, endocrine disruptors; ER, estrogen receptor; FDA, Food and drug administration; FLCs, fetal leydig cells; GD, gestation day; GnRH, gonadotrophin releasing hormone; GPR, G protein-coupled receptor; GSH-Px, glutathione peroxidase; Insl-3, insulin-like 3; KiSS-1, kisspeptin-1; LBW, low birth weight; MBP, mono-n-butyl phthalate; MCIOP, mono (carboxy-isooctyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MHINP, mono (hydroxy-isononyl) phthalate; MiBP, mono-isobutyl phthalate; MINP, mono-isononyl phthalate; MINP-G, mono-isononyl phthalate glucuronide; MOINP, mono (oxo-isononyl) phthalate; NOAEL, No observable adverse effect level; PCOS, polycystic ovary syndrome; PND, postnatal day; PNW, postnatal week; PPARα, peroxisome proliferator-activated receptors; PT, premature thelarche; PVAP, polyvinyl acetate phthalate; ROS, reactive oxygen species; SCC, side chain cleavage enzyme; SOD, superoxide dismutase; AtAR, steroidogenic acute regulatory protein.

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1. Introduction

Humans are exposed to certain persistent environmental chemicals, pesticides, heavy metals, solvents especially organic solvents, synthetic chemicals, plasticizers, illicit drugs, tobacco smoking/chewing, drinking alcohol etc. Some of these are reported to have reproductive toxic potential for both the sexes which might depend upon the dose, duration, and time of exposure to the toxicants as well as host factors such as age, sex, immunity, heredity etc., including their role in reproductive toxicity. Phthalates are just one of the many classes of chemicals that have been reported to have estrogenic or anti-androgenic properties. The approximate consumption of phthalates in the year 2017 was found to be ~ 65% of the world plasticizer consumption, which is expected to be ~ 60% of world's consumption by the year 2022. This might be due to rapid consumption growth for non-phthalate plasticizers in recent years [1]. About three million metric tons of phthalates are produced every year, worldwide [2,3]. Since phthalates are not chemically bound to the plastic matrix, they can easily leach out from phthalate containing products to contaminate the environment [4]. Many sustained or controlled releases drugs (enteric coated tablets) contain cellulose acetate phthalate (CAP), dimethyl phthalate (DMP), dibutyl phthalate (DBP), diethyl phthalate (DEP) and polyvinyl acetate phthalate (PVAP) [2,5,6]. Food and drug administration (FDA) has approved these compounds as excipients with specified amounts for each formulation and route of entry [2,6].

Bioaccumulation of phthalates can occur in medicinal and food plants due to growing of these plants in phthalate contaminated waste water [7]. Young children suck and chew the toys as well as teether containing phthalates, so that they can extract and ingest some quantities of phthalates while chewing these materials. Di-isononyl phthalate (DINP) may be risky for those young children who frequently kept toys (plasticized with DINP) for ~75 min/day or more in their mouth [8]. Based on the available information on rodents and some from human studies, there are health concerns including developmental and reproductive toxicity in human with regard to exposure to phthalates.

It is known that phthalate esters generally consist of a di-ester structure having benzenedicarboxylic acid head group linked to two ester side chains [9]. As shown in Table 1, the phthalate esters panel HPV testing group has categorized three types of phthalates: i.e. low molecular weight phthalates, transitional phthalates and high molecular weight phthalates. Low molecular weight phthalates were defined as those produced from alcohols with straight-chain carbon backbones of ≤C3 [i.e. di-methyl phthalate (DMP), diethyl phthalate (DEP), di-alkyl phthalate (DAP), di-methylethyl phthalate (DMEP), di-isobutyl phthalate (DIBP) etc]. High molecular weight phthalates were defined as those produced from alcohols with straight-chain carbon backbones of ≥C7 or ring structure [i.e. di-isononyl phthalate (DINP), di-nonyl phthalate (DNP), di-isodocyl phthalate (DIDP) etc.]. Transitional phthalates were defined as those are produced from alcohols with straight-chain carbon backbones of C4–6 [i.e. dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-(2-ethylhexyl) phthalate (DEHP) etc.]. Low molecular weight phthalates such as DEP are used as a solvent and fixatives in fragrances; DMP in hair sprays to avoid stiffness of hairs, while the high molecular weight phthalates are used as plasticizers. The transitional phthalates such as DBP are used as a plasticizer in nail polishes to reduce cracking and making them less brittle [10]. They are also used as a solvent [11]. Owing to their day-to-day widespread use, application and ubiquitous nature, the present review is written with the view to look the reproductive toxic potential of phthalates. Thus, this review provides literature survey on phthalate structures, clinical and experimental studies on male and female toxicity, and oxidative stress, including mechanism of phthalate action in both male and female reproductive systems.

2. Data collection

The literature was collected through examining various data resources such as PubMed, Google, Toxnet, and through books and journals pertaining to phthalate exposure and reproductive health. In this review, we attempted to analyse the reproductive toxicity data of phthalates in adults, in utero-treated and developing animals and probable mechanism behind the reproductive impairments and possible implication of exposure to phthalates and human reproduction. This review is divided into different sections based upon the effect of phthalates on male and female reproductive endpoints and on development and pregnancy outcomes. The chemical structures of major phthalate compounds are provided in Table 1, whereas available clinical and experimental data are summarized in Tables 2–5.

3. Phthalate exposure and male reproduction

The effect of any compound on male reproduction can be assessed by determining the adverse effects of compound of interest on the male reproductive system, accessory organs, hormonal balance, time-to-pregnancy, pregnancy outcomes etc. Generally, effects like death, structural malformations or reduced weight of the foetuses are markers of developmental toxicity. While impairment in the reproducing capacity of in utero treated animals or the mature animals denote reproductive toxicity [12]. Animal studies showed the existence of an association between some phthalates and testicular toxicity when exposure to these compounds takes place during prenatal development. This has generated a huge public concern during the past few decades. Exposure to anti-androgenic compounds/chemicals during sexual differentiation results in reproductive tract malformations in mammals, since reproductive system is very sensitive to such chemicals during this period. Common reproductive effects such as decreased fertility and testis weight and alterations in accessory sex organs appeared to be predominantly associated with the transitional phthalates [11]. Thus, many phthalates affecting male reproductive development have been linked to the phthalate syndrome, when given to pregnant rats during in utero sexual differentiation. Perinatal administration of fungicides (vinclozolin and procymidone)or phthalate compounds like DEHP and BBP results in induction of malformations in male rats due to anti-androgenic activity and demasculinize the males by inhibiting the production of fetal testicular testosterone resulting in decreased anogenital distance (AGD), nipple retention, undescended testes, hypospadias, agenesis of epididymis and small to absent accessory sex glands [13].

The data suggest that among the higher molecular weight phthalates (≥ C7), DINP produces some minor effects on development of male reproductive tract at higher doses and among low molecular weight phthalates (≤ C3), DMP and DIBP generally induce slight developmental effects only at high doses [11].

Di-methyl, di-ethyl, di-propyl, di-pentyl, and di-heptyl phthalates were given orally to young male rats at doses of 7.2 mmol/kg/day for 4 days [14] and it was found that only di-pentyl and di-heptyl phthalate produced testicular atrophy, induced a marked increase in urinary zinc excretion and showed decline in testicular zinc content. Later, Howdeshell et al. [15] reported a complete loss of litters at doses 300,600 and 900 mg/kg of di-pentyl phthalate (DPP) in dams from gestation day (GD) 8–18. While a statistically significant decrease in testosterone production was found at doses 100 and 200 mg/kg. Di-hexyl phthalate exhibited developmental and reproductive toxicity at 9900 mg/kg/day and 380–1670 mg/kg/day [16].

In a study by Gray et al. [13], 0.75 g/kg DMP, DEP, DEHP, BBP, and DINP were administered orally to dams from GD14 to postnatal day (PND) 3. DMP and DEP were ineffective while, DEHP and BBP reduced pups body weight at PND1, anogenital distance and testis weight. In the BBP, DEHP and DINP treated groups, males displayed female-like areolas/nipples and significant reproductive malformations. These findings

Table 1
Chemical structures of phthalate compounds [11].

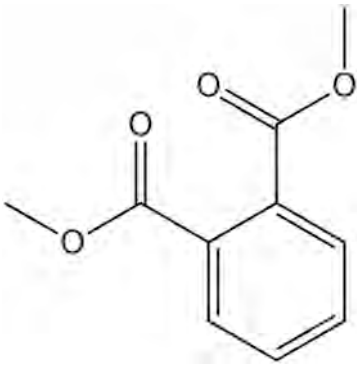
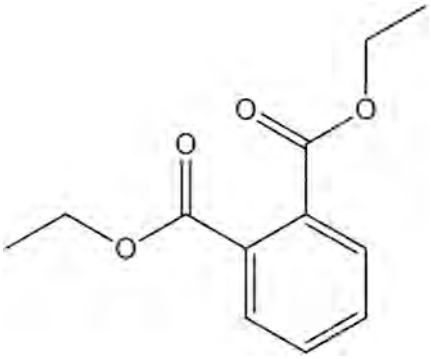
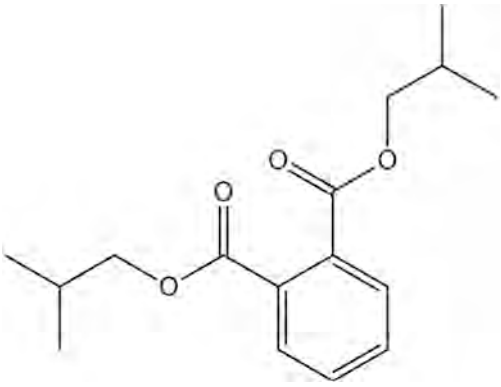
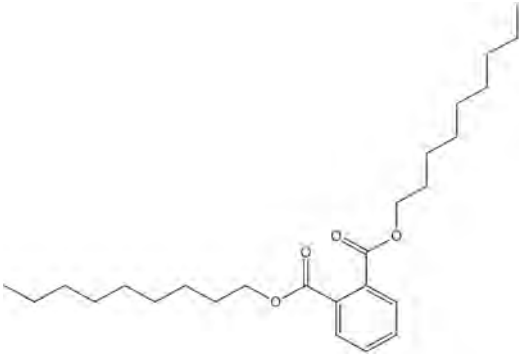
Phthalate	Chemical Structure
Low molecular weight DMP: dimethyl phthalate	
DEP: diethyl phthalate	
DIBP: di-isobutyl phthalate	
High molecular weight DNP: di-nonyl phthalate	

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Table 1 (*continued*)

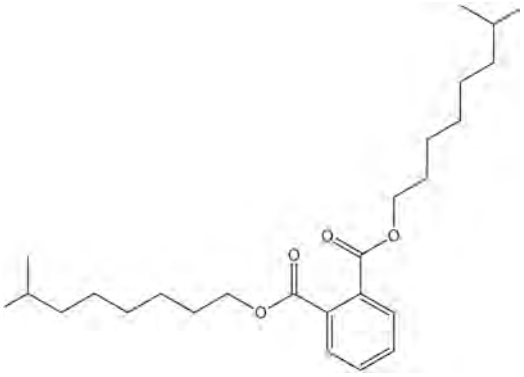
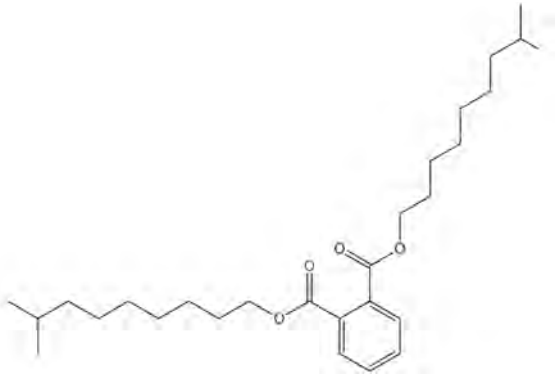
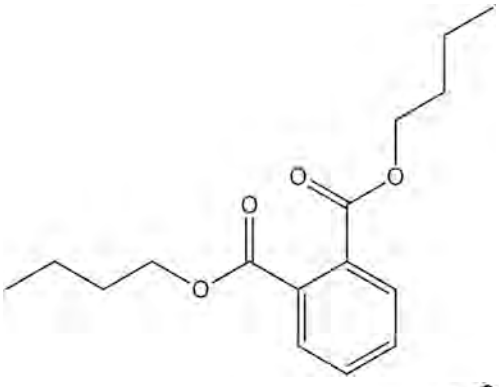
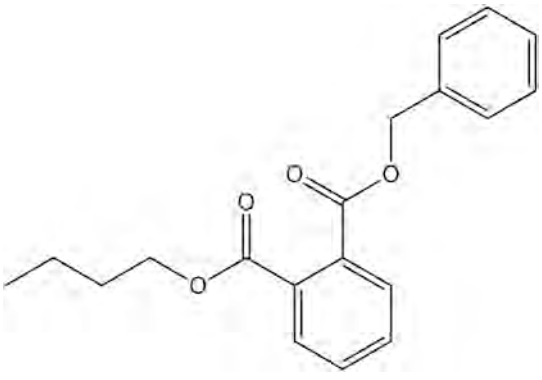
Phthalate	Chemical Structure
DINP: di-isononyl phthalate	
DIDP: di-isodocyl phthalate	
Transitional	
DBP: dibutyl phthalate	
BBP: butyl benzyl phthalate	

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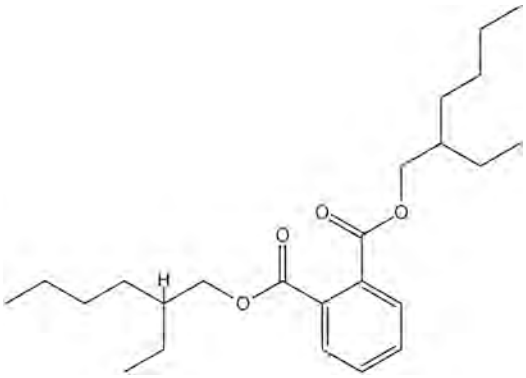
Table 1 (*continued*)

Phthalate	Chemical Structure
DEHP: di-2-ethylhexyl phthalate	

concluded that DEHP, BBP, and DINP altered sexual differentiation but DINP was less toxic than both. Thus, from the studies available it can be stated that low molecular weight phthalates produce least or no reproductive effects.

C5-C10 chain phthalate esters were administered from GD6–15 at doses of 40, 200 and 1000 mg/kg. DEHP showed fetotoxicity, embryo lethality and teratogenicity at higher dose i.e. 1000 mg/kg and no significant effects were observed at 40 and 200 mg/kg. DIDP and three types of DINP showed foetal effects almost significant at 1000 mg/kg [17]. A NOAEL of 276 mg/kg DINP for testicular toxicity was reported in a 104-week chronic rat study based on a reduced testicular weight [18, 19]. Animal studies with di-octyl phthalate showed no adverse effects up to 7500 mg/kg, but adverse effects were seen in high exposure developmental studies [20]. Hannas et al. [21] administered nine phthalates (DEHP, DIHP, DIBP, DBP, BBP, di-cyclohexyl, di-heptyl, di-hexyl, and di-pentyl phthalate) during late gestation (GD14–18). They found that DIBP and di-isoheptyl phthalate (DIHP) decreased testicular testosterone production in the foetus with similar potency to DEHP, whereas DINP was found to be less potent (nearly 2.3-fold). They also reported that the differential effects of in utero DEHP treatment (100, 300, 500, 625, 750, or 875 mg DEHP/kg/day) on epididymal and gubernacular differentiation in male Sprague-Dawley and Wistar rats were likely due to tissue-specific strain differences in the signaling pathways of androgen and Insl-3 [21]. Another study by Ahmad et al. [22] also reported that DBP and BBP exposure from GD14 to parturition might have adverse effects on offspring's reproductive health. In a study by Borch et al. [23], pregnant rats were treated during gestation and lactation with DEHP (300 or 750 mg/kg), DINP (750 mg/kg), and DEHP (300 mg/kg) in combination with DINP (750 mg/kg). By a similar mechanism of action, DINP and DEHP reduced in vitro testicular testosterone production and testosterone levels in testes and plasma of male fetuses at GD21, indicating a synergistic effect of both of the phthalates. Male offspring showed reduced AGD and high nipple retention at PND13 [23].

Clewell et al. [24] treated dams from GD12-PND14 with DINP at 760, 3800, 11,400 ppm DINP and DBP at 7600 ppm in diet. They found a decrease in maternal and pups body weight on PND2 and PND14 at high doses of DINP. Reduced AGD was observed at PND2 and 14, while increased nipple retention and reproductive tract malformations were found on PND49 in DBP treated male pups. DINP (11,400 ppm) reduced AGD on PND14. But no alterations were found in AGD, and nipple retention or reproductive tract malformations on PND49 in the DINP treated groups [24]. Earlier, Waterman et al. [25] administered 100, 500 and 1000 mg/kg DIDP and DINP to rats from GD6–15 to investigate developmental toxicity. Slight maternal and developmental toxic effects were observed at 1000 mg/kg [25].

To determine the impact of dietary exposure of endocrine disruptors (EDs) during brain sexual differentiation, rats were treated with DINP (400, 4000, 20,000 ppm), methoxychlor and genistein from GD15 to PND10 [26]. The highest dose of DINP resulted in minimal degeneration of meiotic spermatocytes and sertoli cells in the testis. In another study [27], dams were treated with 300, 600, 750 or 900 mg/kg DINP from GD7-PND17. DINP caused increased nipple retention and sperm count, and reduced AGD and sperm motility in male offsprings. This study showed that DINP produces anti-androgenic effects on reproductive development, though it is less potent than DEHP, DBP and BBP, thus, emphasizing safety evaluation for DINP. Clewell et al. [28] evaluated the dose response effects of DINP on sexual development of fetal male rat as well as metabolite disposition in the dam and foetus by treating 4 separate groups ($n = 8$) of pregnant dams with 0, 50, 250, and 500 mg/kg of DINP, respectively from GD12–19. Mono-isononyl phthalate (MINP), mono (carboxy-isooctyl) phthalate (MCIOP), mono (hydroxy-isononyl) phthalate (MHINP), mono (oxo-isononyl) phthalate (MOINP), and mono-isononyl phthalate glucuronide (MINP-G) were found in all measured tissues. MCIOP was the major metabolite, followed by MINP, MHINP, MOINP, and MINP-G. Testosterone concentration in the fetal testes was reduced in 250 and 500 mg/kg treated groups. In a Chernoff-Kavlock screening assay, pregnant CD-1 mice were gavage with 4000 mg/kg DIBP from GD6–13 [29]. Out of 50 exposed dams, 27 died and no pregnant dams gave birth to a live litter. Later pregnant wistar rats were treated with 600 mg/kg of DIBP from GD7–19 or 20/21 [30]. Administration of DIBP resulted in significant reduction in AGD in male pups and increased AGD in female pups at GD20/21 along with reduction in body weights of male and female foetuses. Reduction in testicular testosterone production was also noted. In a study [31], pregnant dams were exposed to DIBP from GD7–21. At GD19 or 21, DIBP reduced AGD, testosterone production, testicular insulin-like 3 (Insl-3) expression, steroidogenesis genes and peroxisome proliferator-activated receptors (PPARα) mRNA levels in testis of male offsprings.

To study the developmental toxicity of DIBP, Saillenfait et al. [32] treated dams with 250, 500, 750, and 1000 mg/kg of DIBP on GD6–20. Resorptions and incidence of undescended testes increased significantly at higher doses (750 and 1000 mg/kg). They observed a dose-dependent decrease in fetal weight. The NOAEL of this study was 250 mg/kg DIBP based on decreased pup weight and increased incidence of undescended testes. Later, Saillenfait et al. [33] exposed pregnant rats with 125, 250, 500, 625 mg/kg DIBP and 500 mg/kg DBP, by gavage on GD12–21. No maternal toxicity or reduction in litter size was observed. Reduced neonatal AGD at 250 mg/kg DIBP or higher doses, and dose-related retention of areolas/nipples on PND12–14 were observed in the male offsprings. Delayed preputial separation and undescended testes were observed at 500 and 625 mg/kg DIBP in male offsprings. They concluded that DIBP has adverse effects on the developing male

Table 2
Experimental studies of phthalate compounds on male reproductive system.

Phthalates	Observed effects	References
In utero phthalates exposure: pregnancy outcome and male reproduction		
DBP & BBP treatment from GD14 to parturition	Effects on offspring development, steroidogenesis & spermatogenesis	[22]
760, 3800, 11400 ppm DINP & 7600 ppm DBP through diet from GD12 to PND14	DBP↓AGD & ↑nipple retention, reproductive tract malformations. DINP (11400 ppm) ↓AGD on PND14	[24]
50, 250, & 500 mg/kg DINP from GD12-19	↓T conc. in fetal testes in 250 & 500 mg/kg. NOAEL 50 mg/kg determined on testes testosterone conc. in fetal rat	[28]
DEHP, DIHP, DIBP, DBP, BBP, di-cyclohexyl, di-heptyl, di-hexyl, and di-pentyl phthalate) during GD14-18	DIBP & DIHP ↓T with similar potency to DEHP, whereas DINP was 2.3-fold less potent.	[21]
Dams treated from GD7-PND17 with 300, 600, 750 or 900 mg/kg DINP	↑nipple retention & ↓sperm count, AGD & sperm motility. ↑masculinization of behaviour in females	[27]
Rats exposed from GD7–21 to DIBP, per-fluorooctanoate, butyl paraben, or rosiglitazone	At GD19 or 21 DIBP↓AGD, T, expression of testicular Insl3,steroid-genesis genes & PPARα mRNA	[31]
104-wks chronic rat study with DINP	A NOAEL of 276 mg/kg DINP based on a ↓testicular weight reported	[18,19]
Dams exposed from GD8-18 with 50, 100, 200, 300, 600 & 900 mg/kg Di-pentyl phthalate	A complete loss of litters at doses 300 mg/kg DPP & above. A significant ↓ T at 100 & 200 mg/kg	[15]
Rats treated 125, 250, 500, 625 mg/kg DIBP & 500 mg/kg DBP from GD12–21	DIBP embryotoxic& teratogenic but DIBP slightly less potent than DBP	[33]
Pregnant rats were treated with 600 mg/kg of DIBP from GD7-19 or 20/21	↓AGD in male pups & ↑AGD in female pups at GD20/21; ↓body wt. of male, female foetuses & testicular T	[30]
Dams treated 250, 500, 750, & 1000 mg/kg of DIBP on GD6-20.	Resorptions & undescended testes↑ & fetal wt↓ at high doses.NOAEL 250 mg/kg based on pup wt & undescended testes	[32]
Rats treated in gestation & lactation with DEHP (300, 750), DINP (750), or DEHP + DINP (300+750) mg/kg	↓Fetal testicular T *in vitro* & *in vivo* at GD21. AGD↓ & nipple retention↑ in DEHP exposed male offsprings.	[23]
Rats treated with di-isononyl phthalate from GD15 to PND10	Minimal degeneration of meiotic spermatocytes & sertoli cells	[26]
Di-hexyl phthalate at 9900 mg/kg and 380-1670 mg/kg	Exhibited developmental &reproductive toxicity	[16]
Animal studies with di-octyl phthalate	No adverse effects up to 7500 mg/kg	[20]
0.75 g/kg DMP, DEP, DEHP, BBP, & DINP administered from GD14 to postnatal day 3	DEHP, BBP & DINP altered sexual differentiation. DINP less active than DEHP & BBP. DMP &DEP ineffective	[13]
DIDP & DINP to rats from GD6-15	Slight maternal & developmental effect at 1000 mg/kg	[25]
C5-C10 chains phthalate administered from GD 6-15 at doses of 40, 200, 1000 mg/kg	DEHP showed fetotoxicity, embryo-lethality & teratogenicity at 1000 mg/kg. DIDP & DINP showed foetal effects of borderline significance at 1000 mg/kg	[17]
In a Chernoff-Kavlock assay, mice gavage with 4000 mg/kg DIBP from GD6-13	No pregnant dams gave birth to a live litter and 27/50 exposed dams died.	[29]
Di-methyl, di-ethyl, di-propyl, di-pentyl, di-heptyl phthalates orally to male rats at 7.2 mmol/kg/day for 4 days	Di-n-pentyl and di-n-heptyl phthalate ↑urinary zinc excretion with ↓testicular zinc concentration	[14]
Testicular dysgenesis		
Rats exposed from GD7–21 to DIBP, DMP & DEP	DIBP ↓in plasma leptin level in male & female offsprings. DIBP ↓AGD, T, expression of Insl-3 & steroidogenesis related genes in males.↓PPARα mRNA at GD19 in testis & liver. While DMP & DEP have no effect	[31]
Single dose DBP exposure on GD18 & euthanized on GD19.	Malformed epididymis, hypospadias, cryptorchidism, retained thoracic nipples due to ↓expression of steroidogenic enzymes &↓T.	[95]
DIBP exposure male foetuses	↓testicular T ex vivo & testosterone level in testes & plasma, ↓AGD, clustering of small leydig cells & vacuolisation of sertoli cells in male foetuses.	[90]
Anti-androgenic effects of transitional phthalates	Produce anti-androgenic effects by inhibiting production of foetal T & Insl-3.Gestational exposure to BBP, DBP or DEHP induced a decrease in expression of Insl-3 in rat fetal testes. Serum T was reduced following gestational treatment with DEHP, BBP or DBP	[87, 91–94]
Oxidative stress		
DBP administered 100, 250 & 500 mg/kg/day for 2 wks.	↓Epididymal alpha-glucosidase & glutathione peroxidase activity at500 mg/kg. ↓SOD activity & ↑MDA in epididymal tissue at 250,500 mg/kg DBP	[83]
Rats treated orally with 50, 250, 500 & 1000 mg/kg DIBP for 8 wks.	↓SOD, GSHPx activities &↑MDA, 8-OHdG indicating oxidative stress &DIBP can also decline antioxidative enzyme activities resulting in oxidative damage to tissues.	[98]
Induced hyperthyroidism in pubertal male rats by injecting triiodothyronine with a simultaneous administration of DBP to normal or hyperthyroid rats.	↑Serum T_3 levels & ↓serum TSH level in hyperthyroid rats. Hyperthyroidism can cause a change in the expression level of PPARγ in testes & may increase the levels of oxidative damage induced by the metabolic activation of DBP.	[100]
DBP administered 250, 500, & 1000 mg/kg to rats	↓testis SOD activities at 1000 mg/kg. ↓GSHPx activities in serum & GSH levels in testis, but ↑GSHPx activities in testis after 2-wk DBP exposure, After 4-wk exposure, ↑alkaline phosphatase activities, ↓SOD activities in both serum& testis.	[101,102]
Oral administration of DEHP to 4-5 weeks old rats	↑ROS, ↓GSH &ascorbic acid in testes leading to apoptosis & testicular atrophy	[86]
Limb bud cells extracted from rats on gestation day 12.5 & treated with DBP or MBP	DBP and MBP induced developmental toxicity in rat embryonic limb bud cells which exerted through oxidative stress.	[103]

↓- decrease: ↑-increase: T-Testosterone

reproductive tract, at maternally toxic doses since it is embryotoxic and teratogenic but slightly less potent toxic than DBP. Above studies raise a concern about the use of DIBP as a substitute for DBP since DIBP might also possess similar testicular and developmental effects as DBP and DEHP [33].

4. Clinical studies on male reproduction

There are several clinical reports which indicated that some of the phthalates have adverse effects on human male reproduction, but the data are sparse and inconsistent as human are exposed to number of other chemical and physical factors and some of them might be associated with adverse effect on reproduction. Duty et al. [34] studied whether environmental levels of phthalates are associated with altered semen quality in humans and found a dose-response relation between mono-butyl phthalate, sperm motility and concentration. Further, a dose-response relation was also observed between mono-benzyl phthalate and sperm concentration. Later, Hauser et al. [35] established findings with mono-butyl phthalate and mono-benzyl phthalate but did not find association between semen parameters with three metabolites

Table 3
Clinical studies in males with respect to exposure to phthalate compounds.

Phthalates	Observed effects	References
Impact of DEHP metabolites on male reproduction	A negative association between DEHP metabolites & testosterone levels.	[38]
Associations of phthalate with semen quality and reproductive hormones	↓semen volume [MBP, MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-oxohexyl) phthalate], ↓sperm curvilinear velocity [MBzP, MEHP] &↓straight-line velocity [MBzP, MEHP, % MEHP], & ↑abnormal sperms [MBzP]. No links between seminal phthalate metabolites & reproductive hormones.	[37]
Associations between urinary phthalate metabolites, sperm acrosin activity & insulin like-factor 3 (Insl-3), in adult men	Insl-3 negatively associated with MEHP. Acrosin activity negatively linked with MBP, MiBP, MEHP & %MEHP. MBP &MiBP also negatively linked with total testosterone, free androgen index, free testosterone, LH & sperm morphology & positively related with DNA fragmentation. A negative connection between % MEHP & sperm motility.	[36]
Environmental phthalates and semen quality in humans	No associations between semen quality & three metabolites of DEHP.	[35]
Urinary DBP, DIBP & other phthalates in women &male genitals.	Concentration of urinary DIBP was inversely related to anogenital index.	[39]
Environmental phthalates & semen quality in humans	Relation between mono-butyl phthalate & sperm motility & concentration. A dose-response relation between mono-benzyl phthalate &sperm concentration.	[34]

↓- decrease

of di-2-ethylhexyl phthalate (DEHP).

Pan et al. [36] investigated association between urinary phthalate metabolites, sperm acrosin activity, and Insl-3 expression, in adult men. They found that Insl-3was negatively associated with mono-2-ethylhexyl phthalate (MEHP) and % MEHP [% of MEHP to all di (2-ethylhexyl) phthalate (DEHP) metabolites]. In case of acrosin activity, negative association was found with mono-n-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), MEHP and %MEHP. While MBP and MiBP were also negatively linked with total testosterone, free androgen index, free testosterone, LH, sperm morphology and positively associated with DNA fragmentation index. A negative association was observed between % MEHP and sperm motility. Later, Wang et al. [37] reported that semen phthalate metabolites were significantly associated with decrease in semen volume [MBP, MEHP, mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP)], sperm curvilinear velocity [monobenzyl phthalate (MBzP), MEHP, the percentage of DEHP metabolites excreted as MEHP (%MEHP)], and straight-line velocity (MBzP, MEHP, %MEHP), and also associated with high abnormal head and tail percentage with MBzP. These associations were significant after adjustment for multiple testing. However, semen phthalate metabolites and serum reproductive hormones relationship was not noteworthy. Very recently, Hoyer et al. [38] reported a more consistent negative association between metabolites of DEHP and testosterone. While, inconsistent results were reported between higher urinary DEHP metabolite levels with elevated damaged DNA spermatozoa and a decline in sperm motility and count.

Swan et al. [39] found an association between levels of DBP, DIBP and other phthalates in the urine of pregnant women and alterations in the genitals of male infants which were consistent with phthalate related syndrome previously reported in prenatally exposed rats. They showed that concentration of urinary DIBP was inversely related to anogenital index in male children. The available clinical data suggests that some of the phthalates may cause multiple adverse results on male reproduction; lack of reproductive toxicity of some of the phthalate compounds may indicate a difference in spermatotoxicity of phthalates.

5. Phthalate exposure and female reproduction

Considerable toxicity data of phthalates on male reproduction are available as compared to female. Thus, it is generally considered that female reproduction is less sensitive to phthalates than male; however, some studies have shown that some phthalates may also have a significant effect on female reproduction. McLachlan et al. [40] reported that human development could also be feminized by exposure to estrogenic chemicals. Estrogen is the key hormone in the initiation (puberty) and the end (menopause) of reproductive life in women and thus it has considerable importance in women's health. Later, Martino-Andrade and Chahoud [41] reviewed the data on phthalates and mentioned that the reproductive effects of phthalate compounds are well characterized in adult animals, with gonadal injury observed after high dose exposure, and results of various trans-generational studies indicate that the reproductive system of developing animals is vulnerable to certain phthalates. They also reported that high phthalate doses can adversely affect adult and developing female rats.

A few experimental studies are available on the effects of phthalates on female reproduction. Grayet al. [42] reported that in contrast to the male rat, it is generally felt that reproduction in female rat is less sensitive to phthalate. They found that administration of DBP to female rats from weaning, through puberty, mating, and gestation disrupts pregnancy maintenance at dose levels like those that affect testis function in rats. Administration of 500 and 1000 mg DBP/kg to female rats induced mid-pregnancy abortions. The percentage of females delivering live pups was reduced by more than 50% and 90% at 500 and 1000 mg/kg dose, respectively, whereas the ages at vaginal opening and first estrous, estrous cyclicity, and mating indices were not significantly affected. Earlier, Davis et al. [43] studied toxic potential of DEHP in adult female cycling rats. Animals were dosed daily with 2 g/kg DEHP for 1–12 days. DEHP exposure resulted in prolonged estrous cycles and delayed ovulations significantly by altering natural ovulation times. They concluded that exposure to DEHP resulted in hypoestrogenic ovulatory cycles and polycystic ovaries in adult female rats. Later, Lovekamp-Swan and Davis [44] reported that in vivo, DEHP (2 g/kg) causes decreased serum estradiol levels, prolonged estrous cycles, and no ovulations in adult, cycling rats. In vitro, MEHP; metabolite of DEHP decreases granulosa cell aromatase RNA message and protein levels in a dose-dependent manner. They also mentioned that MEHP acts on the granulosa cell by decreasing cAMP stimulated by FSH and by activating the PPARs, which leads to decreased aromatase transcription (Fig. 1). Thus, DEHP, through its metabolite MEHP, acts through a receptor-mediated signalling pathway to suppress estradiol production in the ovary, leading to anovulation. Later, it was reported by Ma et al. [45] that DEHP may advance the onset of puberty and alter post pubertal reproductive functions of rats. Above studies suggest that DEHP affects the female reproductive function. Recently, it has been reported that BBP, DBP, DIBP and DINP might not have in vivo estrogenic potential based on 3-day utero-trophic and 20-day pubertal female assay [46,47].

6. In utero exposure to phthalates and pregnancy outcome

Several reports indicate that in utero exposure to phthalate compounds had adverse effect on pregnancy outcome. Available experimental studies indicated that in utero exposure to phthalates led to various developmental and reproductive impairments in the offsprings.

Table 4
Experimental studies of phthalate compounds on female reproduction system.

Phthalates	Observed effects	Reference
Phthalates treatment to female		
DEHP 5, & 25 mg/m^3 to female rats for 6 h/day (5 days/wk) from postnatal days 22 to 41	DEHP may advance the onset of puberty & alter post-pubertal reproductive functions of rats.	[45]
Studied toxic potential of 2g/kg DEHP in 1 to 12 days in regularly cycling rats	Long estrous cycles & delay ovulation Suppress serum E2 caused secondary ↑in FSH & did not stimulate the LH.	[43]
Estrogenic potential- In vitro studies		
Yeast-based assay to estimate the estrogenic potential	DEP, DBP & BBP show estrogenic activity But DMP & DOP did not show.	[120]
DEHP tested by human breast cancer Estrogen-dependent MCF-7 cell proliferation assay.	DEHP enhanced the proliferation of human breast cancer MCF-7 cells in vitro indicating an estrogenic activity.	[121]
BBP, DBP, di-n-octyl phthalate (DOP), &di-nonyl phthalate (DNP)	BBP & DBP -↑ estrogenic activity. DOP &DNP showed weak activity.	[122]
BBP, DBP, n-butyl phthalyln-butyl glycolate, DEHP & benzyl salicylate	↑ Proliferation of MCF-7 cells indicating estrogenic potential of these.	[123]
Estrogenic activities of phthalate di & monoesters by human breast cancer MCF-7 cell proliferation assay	Among 19 compounds tested, di-cyclohexyl phthalate, DEHP & BBP found estrogenic activities.	[124]
Anti-estrogenic activities by the suppression of cell proliferation in presence of 10^{-11} M 17β-estradiol.	Mono-n-pentyl phthalate, mono-cyclohexyl phthalate, mono-benzyl phthalate, mono-isopropyl phthalate & BBP have anti-estrogenic activities.	
DBP, BBP, DINP, DEHP, di-hexyl (DHP), di-isoheptyl, di-n-octyl, &di-isodecyl phthalate	DBP, BBP, & DHP exhibit weak ER-mediated activity in some of in vitro assays.	[125]
BBP, DBP,DIBP, diethyl phthalate (DEP), & di-isiononyl phthalate (DINP)	The relative estrogenic potencies of these descended in the BBP > DBP > DIBP > DEP > DINP.	[126]
Estrogenic potential - In vivo studies		
Estrogenic potential of DIBP & DINP *in vivo*	DIBP & DINP do not have estrogenic potential with 3-day utero-trophic & 20-day pubertal female assay.	[47]
Estrogenic potential of DBP & BBP *in vivo*	DBP & BBP do not have estrogenic potential with 3-day utero-trophic & 20-day pubertal female assay.	[46]
Estrogenic potential of BBP,DPB, DOP & DNP tested *in vivo*	Not increased uterus wt in immature rats in any phthalate treated animals.	[122]
DEHP, DBP, BBP, DHP, DOP, DINP, di-isoheptyl, di-isodecyl phthalate compounds tested for *in vivo* estrogenic response	None of the eight-phthalate esters tested showed *in vivo* estrogenic potential based upon utero-trophic & vaginal cornification assays.	[125]
In utero exposure to phthalates and pregnancy outcomes		
DEHP in pregnancy & lactation on development & function of pituitary-gonadal axis in mice male & female offsprings	DEHP acts on multiple pathways involved in maintaining steroid homeostasis & alter estrogen synthesis in both sexes.	[48]
BBP (120 or 500 mg/kg/day) from day 10 post-conception to delivery) to rats	Delayed vaginal opening& high dose affects architecture & proliferative index of post-natal mammary gland.	[49]
Evaluated DEHP induce damage in sexual development of female offspring of rats after maternal exposure	Exposure to DEHP in utero from GD 12-17 can result in abnormalities of sexual development like time to vaginal opening & atresia.	[54]
100 mg/kg/d of DBP GD 12-20 & assess reproductive outcome	DBP did not disturb the reproductive development or function of female rats.	[50]
500 & 1000 mg DBP/kg/day, from weaning, through puberty, mating & gestation to female rats	Induced abortions. The % of females delivering live pups↓ by more than 50% & 90% at 500 & 1000 mg/kg.	[42]
Rats treated GD 6 to lactation day 22. Low 0.015 to 1.215 & high doses 5 to 405 mg DEHP/kg/day	Delay in vaginal opening at 15 mg DEHP/kg/day & delay in the age at first estrous at 135 & 405 mg DEHP/kg/day.	[52]
Embryonic toxicity		
Rats treated BBP 250, 500, 750, & 1000 mg/kg from GD0-8 & pregnancy outcome determined	↑Pre-implantation loss at 1000 mg/kg. Post-implantation loss at 750 mg/kg & above. ↓Uterine decidual growth in pseudo-pregnant at 750 mg/kg & above.	[73]
Treatment with BBP from GD13–15	0.75 & 1.0 g/kg BBP was teratogenic.	[74]
Rats treated at dose of 0.5, 0.75 or 1.0 g BBP/kg from GD7–15	Maternal lethality &complete resorption in 1.0 g/kg. ↑Embryo-fetal death &↓fetal wt at 0.75 g/ kg.	[75]
Single dose of di methoxy ethyl phthalate to rats during gestation	Embryopathy manifested 12-79% of foetal deaths & resorptions.	[71]
Mice treated with 0.1, 0.2, 0.4, & 1.0% DEHP, and DBP by food during gestation	↓Maternal wt gain & ↑resorption. Implanted ova died at 0.4, 1.0% of DEHP. ↑Malformation at 0.2% of DEHP & 1.0% of DBP.	[72]

↓- decrease: ↑-increase

Pocar et al. [48] examined the effects of DEHP exposure in mice throughout pregnancy and lactation on the development and function of the pituitary-gonadal axis in male and female offsprings. They stated that, in maternally exposed male and female mice, DEHP acts on multiple pathways involved in maintaining steroid homeostasis. Additional exposure of lactational DEHP may alter estrogen synthesis in both sexes. Prenatal BBP exposure induced delayed vaginal opening and post-natal mammary gland changes i.e. alterations in architecture and proliferative index of the mammary gland morphology [49]. In another study, rats were treated with DBP (100 mg/kg/day) from GD12 to GD20 for evaluation of reproductive outcomes, reproductive development and function in F1 female offsprings and found that all parameters were comparable with control group, except a remarkable increase in the fetal weight in the treated group. They concluded that DBP did not disturb or alter the reproductive development or function of female rats [50]. Earlier, Lee et al. [51] evaluated developmental toxicity of DBP exposure from late gestation to lactation. Female offsprings showed a slight non-significant delay in the pubertal onset and the relative pituitary weight was found to decrease after 10,000 ppm exposure at postnatal week (PNW) 11, and from 200 ppm at PNW 20. Thus, developmental exposure to 10,000 ppm DBP affected female sexual development involving pituitary function since the proportion of FSH-positive cells increased in the pituitaries at PNW 11of both the sexes.

Grande et al. [52] conducted a study on DEHP. Female rats were treated daily with DEHP from GD6 to PND22 with the low doses i.e. 0.015–1.215 mg, and the high doses 5–405 mg DEHP/kg/day. They neither observed any maternal toxicity nor any alterations in nipple development and anogenital distance. They observed that 15 mg and above 15 mg DEHP caused a significant delay in the age at vaginal opening and135 and 405 mg DEHP caused a delay in the age at first estrus. Piepenbrink et al. [53] also mentioned that, in utero exposure to DEHP alters some developmental parameters i.e. anogenital distance without any persistent effect on the immunity. Later, Ding et al. [54] evaluated the ability of DEHP in inducing damage in sexual development of female rat offsprings. They reported that exposure to DEHP from GD 12–17 resulted in alterations in the age at vaginal opening and atresia.

Table 5
Clinical studies in females with respect to exposure to phthalate compounds.

Phthalates	Observed effects	References
Study on MEHP and pregnancy loss	Mono-ethylhexyl phthalate link with an elevated pregnancy loss.	[58]
Plasma phthalate esters in women with endometriosis	↑ MEHP &DEHP in advanced-stage endometriosis.	[59]
Urinary level of phthalates and pubertal gynecomastia	Significantly higher in pubertal gynecomastia group, girls with thelarche & in precocious puberty.	[70]
Association between phthalate esters and PCOS	PCOS patients had significantly higher level of DEP and DBP.	[63]
Urinary phthalate metabolite concentrations in relation to reported history of endometriosis & uterine leiomyomata	ORs of urinary MBP 1.36 for endometriosis, 1.56 for leiomyomata, & 1.71 for combined. ORs for MEHP 0.44 for endometriosis, 0.63 for leiomyomata & 0.59 for combined.	[61]
Assessed the association between urinary conc. of phthalate metabolites & endometriosis	No significant links between endometriosis & urinary creatinine-adjusted phthalate monoester.	[62]
AF & maternal urine samples tested for oxidative metabolites of DBP, DIBP, BBP and DEHP	Some phthalates or metabolites reach human fetus, which affect fetal health, two carboxy metabolites of DEHP showed highest levels.	[56]
Phthalates levels and New-born Chinese	DBP & DEHP in utero exposure links with LBW.	[57]
Link between PCBs & phthalate esters (PEs) & endometriosis	PCBs & PEs may be instrumental in the aetiology of endometriosis.	[60]
Determined pollutants in serum of Puerto Rican girls with thelarche	68% thelarche patients with ↑DMP, DEP, DBP, DEHP & MEHP.	[65]

↑-increase

7. Clinical studies on female reproduction

A few reports claim detectable level of certain phthalates or their metabolites in the amniotic fluid (AF) indicating possible fetal exposure to these compounds. Silva et al. [55] found considerable amount of monoethyl phthalate (MEP), MBP, and MEHP in human AF, suggesting their presence in the human fetal environment in the second trimester early stages when reproductive differentiation takes place. Later, Wittassek et al. [56] collected AF and corresponding maternal urine (MU) samples, and found that the concentrations of metabolites of DBP, DIBP, BBP and DEHP were generally much higher in the MU samples as compared to the AF samples. They suggested that several phthalates or their metabolites might reach the human foetus affecting foetal health. Further, in a report by Zhang et al. [57] considerably higher amount of phthalates were detected in low birth weight (LBW) babies as in China new-born babies are very frequently exposed to phthalates. Prenatal DBP and DEHP exposures were associated with LBW in a dose-dependent manner which may be a considered as a risk factor for LBW.

In humans, MEHP was associated with a high odds ratio (ORs) of pregnancy loss as reported [58]. Kim et al. [59] conducted a prospective case-control study and found that the concentrations of DEHP and MEHP were significantly higher in advanced-stage endometriosis subjects, which supports that exposure to phthalates play a significant role in the formation of endometriosis. Earlier, Reddy et al. [60] also found that polychlorinated biphenyls (PCBs) and phthalate esters (PEs) might be instrumental in the aetiology of endometriosis. Further, Weuve et al. [61] conducted a cross-sectional study of urinary phthalate metabolite concentrations in relation to reported history of endometriosis and uterine leiomyomata. After diagnosis, endometriosis and leiomyomata were found to be about 7% and 12%, respectively. The ORs comparing the highest versus lowest, three quartiles of urinary MBP were 1.36 for endometriosis, 1.56 for leiomyomata, and 1.71 for both. The corresponding ORs for MEHP were 0.44 for endometriosis, 0.63 for leiomyomata, and 0.59 for both. However, the research of Itoh et al. [62] does not support the hypothesis that higher urinary concentrations of phthalate metabolites are associated with the risk of endometriosis as no significant association between endometriosis and urinary creatinine-adjusted phthalate monoester was found.

Xu et al. [63] assessed the association between phthalate esters and polycystic ovary syndrome (PCOS). They found that PCOS patients had significantly higher level of DEP and DBP than control, suggesting an association of phthalates with PCOS. Further, Job-exposure matrix based maternal occupational exposure to phthalates was associated with prolonged time to pregnancy (OR 2.16, 95% CI 1.02–4.57) and exposure to pesticides was associated with LBW (OR 2.42, 95% CI 1.10–5.34). They concluded that maternal occupational exposure to phthalates and pesticides has adverse effects on fertility and pregnancy outcomes [64].

Earlier, Colon et al. [65] determined pollutants in the serum of Puerto Rican girls with premature thelarche. A significantly high level of phthalates (DMP, DEP, DBP and DEHP and its major metabolite MEHP) were identified in 28 (68%) samples from thelarche patients whereas only one control subject showed significant levels of di-isooctyl phthalate. This suggests a possible association between plasticizers and the cause of premature breast development in humans. Later, Chou et al. [66] conducted a case-control study by recruiting girls in early puberty, with premature thelarche (PT) and central precocious puberty (CPP). The mean urine levels of monomethyl phthalate (MMP) were significantly higher in the PT group. Recently, Durmaz et al. [67] found a significantly higher concentration of MEHP in the urine of girls with premature thelarche. These data suggest that phthalate might be one of the environmental causes of early puberty in girls. However, Lomenick et al. [68] suggested that phthalate exposure is not associated with precocious puberty in female children. McKee [69] also reported relationship between phthalate exposure and early thelarche seems highly unlikely, in part because the reported exposure levels do not seem plausible on phthalate exposure, and phthalates do not influence the timing of female sexual development in laboratory studies. Thus, these results need to be substantiated with more studies.

In a review by Jurewicz and Hanke [70], it was strongly emphasized that phthalates increase allergy and asthma risk. Decreased (fewer masculine) composite score in boys and quality of alertness among girls indicated that neurodevelopment of children is adversely affected by phthalates. Inconsistent results revealed a negative impact of phthalates on gestational age and head circumference. Further, exposure to phthalates adversely affected luteinizing hormone, free testosterone, and sex hormone-binding globulin levels, thyroid function and anogenital distance. Epidemiological studies suggest that phthalates might affect reproductive outcome and children health since the urinary levels of phthalates were remarkably higher in the pubertal gynecomastia group, girls with premature thelarche, and precocious puberty [70].

8. Embryonic toxicity

A single intraperitoneal injection (0.6 ml/kg) of di-methoxyethyl phthalate (DMEP) was given to rats during gestation. In phthalate treated rats, embryopathy was manifested by 12–79% of fetal deaths and fetal resorptions. Fetotoxicity was expressed by a significant reduction in fetal weights. DMEP caused a congenital malformation of the brain i.e. hydrocephalus interna [71]. Shiota et al. [72] treated pregnant mice with DEHP and DBP in food GD0-parturition. They observed increased resorption rate and death of all the implanted ova at 0.4% and 1.0% level of DEHP. Rate of malformation increased at 0.2% DEHP and 1.0% DBP in term fetuses which was almost significant. Their results suggest that high dose of DEHP and DBP could be teratogenic and embryotoxic in mice.

There is a report on impairment of uterine function by BBP.BBP (1000 mg/kg) resulted in a significant increase in pre-implantation loss

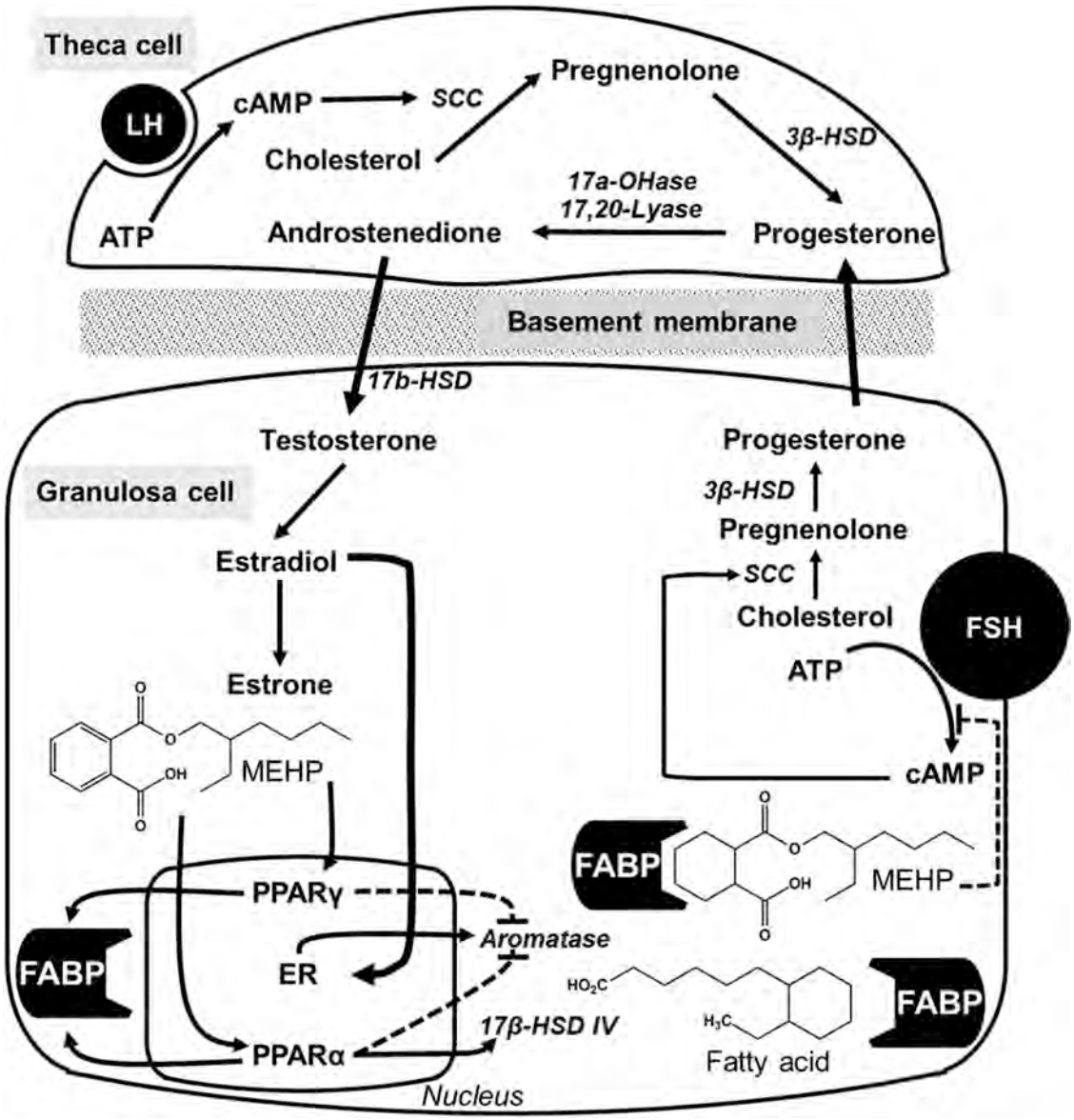


Fig. 1. Proposed model of MEHP interference in the granulosa cell via steroid hormone pathway [44].

while BBP (750 mg/kg and above) resulted in post-implantation loss in females having implantations at that doses. The early embryonic loss due to BBP may be thought in part to be mediated by suppression of uterine decidualization [73]. Earlier, Makoto et al. [74] treated rats at the doses of 0, 0.5, 0.75 or 1.0 g BBP/kg from GD7–15. In the 1.0 g/kg group, high maternal lethality and complete resorption of implanted embryos were observed in the surviving dams. Fetal death rate increased and fetal weight decreased at 0.75 g/kg. A significantly increased incidence of fetal malformations like cleft palate, fused sternebrae and renal pelvis dilatation were mostly observed in the 0.75 g/kg group. Later, they noted that the highest incidence of malformed foetuses occurred after treatment with BBP on days 13–15. It could be concluded that the susceptibility to the teratogenicity of BBP varies with the developmental stage at the time of administration [75]. These data showed that few phthalate compounds such as DMEP, DEHP, MEHP, DBP, and BBP, have embryotoxic potential which might depend upon the dose as well as time of exposure during the pregnancy. Fig. 2 shows the possible pathway leading to embryotoxicity after phthalate treatment in rodents [76].

9. Phthalate induced testicular dysgenesis and oxidative stress

Hormonally active environmental chemicals/compounds generally target the endocrine system which leads to reproductive anomalies [77]. An increase in these environmental contaminants causes disturbances in the pro-oxidant/antioxidant balance of testicular cells leading to impairment of testicular functions thereby activating apoptosis [78]. Physiological levels of reactive oxygen species (ROS) and apoptosis are necessary for the normal functioning of the testes and ovaries, but an imbalance may cause deleterious effects as shown in Fig. 3 [79]. Further, insufficient antioxidant enzymes and increased oxidative stress may attribute to the risk of declining semen quality in human [80].

Steroidogenesis and spermatogenesis take place within the seminiferous tubules and interstitium tissue of the testes. Controlled and maintained levels of ROS play a constructive role in normal testicular function [77]. The testis consists of a very potent antioxidant system comprising of superoxide dismutase (SOD), catalase, glutathione family and several non-enzymatic antioxidants which protect it from the damaging effects of ROS. All this help the testis by counteracting any oxidative stress [81]. Several environmental contaminants affect germ cell, resulting in defective spermatogenesis. Phthalates are among a

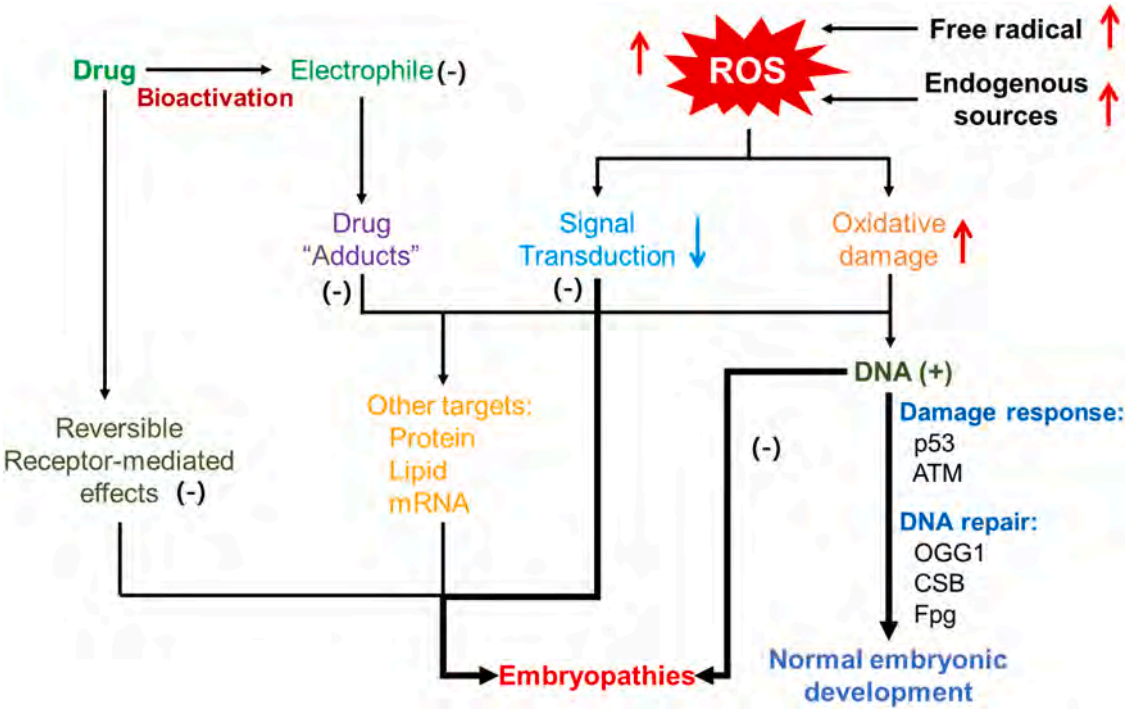


Fig. 2. Possible pathway leading to embryo toxicity after phthalate treatment in rodents [76].

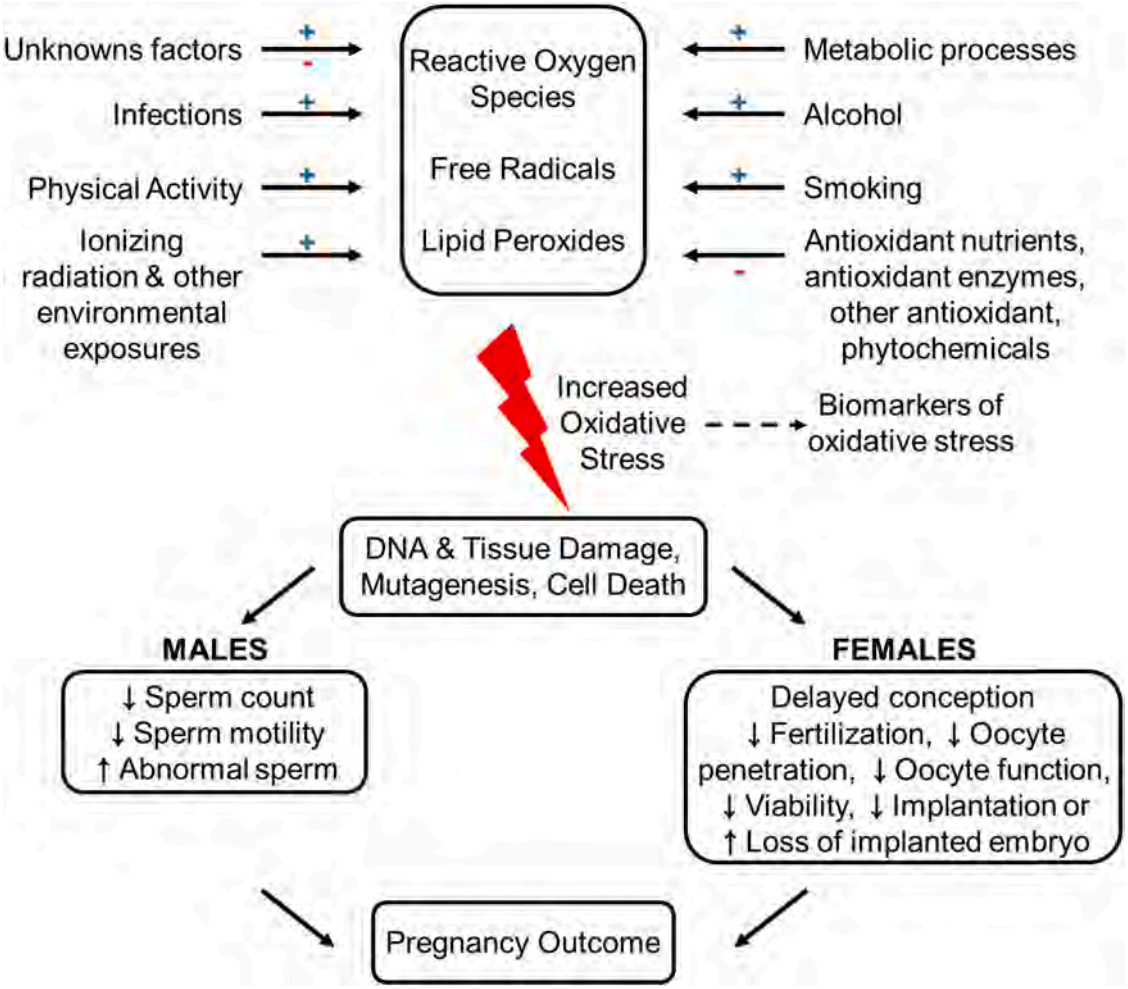


Fig. 3. Deleterious effects of imbalance in ROS and apoptosis [79].

wide variety of environmental toxicants that can compromise male fertility by inducing oxidative stress in the testes, in addition to endocrine disruption. At the level of testes, oxidative stress can disrupt the steroidogenic capability of leydig cells as well as affect the germinal epithelium. Several experimental studies implicate role for oxidative stress in phthalate-stimulated liver tumorigenesis [82], male reproductive toxicity [83,84], and developmental toxicity [85,86].

In the developing fetal testis, toxic phthalate esters target numerous pathways. In fetal leydig cells, molecular pathways associated with synthesis and transport of lipid and cholesterol and with steroidogenesis are reduced, which results in a reduction in testosterone synthesis. Insl-3production by fetal leydig cells is also reduced which might be involved in phthalate-induced cryptorchidism [87]. Insl-3 is involved in testicular descend. A reduction in alpha inhibin production likely plays a role in alteration in sertoli cell maturation and function; this alteration along with phthalate-induced disruption in sertoli-gonocyte interaction likely plays a role in the development of multinucleated gonocytes. Free radical formation is a normal occurrence during steroidogenesis [88] and it is possible that the reduction in expression of genes associated with protecting the cell from oxidative stress such as glutathione transferase and superoxide dismutase are due to a reduction in oxidative stress following reduction of testosterone synthesis [89].

Low molecular weight phthalates such as DMP and DEP have no developmental effects but DIBP has some developmental effects. DIBP, butyl paraben and rosiglitazone were able to reduce the levels of plasma leptin in male and female offsprings at GD19 or 21. DIBP and rosiglitazone also reduced the levels of fetal plasma insulin. DIBP reduced AGD, testosterone production and expression of Insl-3 in testis and steroidogenesis related genes in males. PPARα mRNA levels were also reduced at GD19 in testis and liver by DIBP [31]. DIBP decreased ex vivo testicular testosterone production, testosterone levels in testis and plasma, decreased AGD and induced pathological changes in the testes including clustering of small leydig cells and vacuolisation of sertoli cells in the male foetuses [90].

Transitional phthalates produce anti-androgen effects by inhibiting production of fetal testosterone and Insl-3. Gestational exposure to BBP, DBP or DEHP induced a decrease in expression of Insl-3 in rat fetal testes [87] perhaps explaining the increased incidence of cryptorchidism. There are reports on reduced serum testosterone following gestational treatment with DEHP, BBP or DBP [91–94]. Prolonged in utero DBP exposure results in feminized phenotypic symptoms like malformed epididymis, hypospadias, cryptorchidism, retained thoracic nipples etc. which are likely due to decreased expression of steroidogenic enzymes and reduction in the testosterone biosynthesis [95].

Epidemiologic studies have shown relationships between biomarkers of phthalate exposure and increased levels of malondialdehyde (MDA), and 8-hydroxy-2-deoxy guanosine (8-OHdG) [96,97]. In a study by Ma et al. [98], SOD and glutathione peroxidase (GSH-Px) activities in DIBP treated groups were significantly lower while MDA and 8-OHdG contents were significantly higher, indicating that oxidative stress induced by DIBP can decrease antioxidative enzyme activities resulting in oxidative damage in tissues. Hong et al. [96] reported that polyaromatic hydrocarbons, volatile organic compounds, bisphenol-A and phthalate exposure are associated with oxidative stress in urban adult populations.

In a study carried out by Zhou et al. [83], a significant decrease in the epididymal weight and in the activity of epididymal alpha-glucosidase, GSH-Px, and SOD was found in rats exposed to DBP (500 mg/kg). While the level of MDA increased significantly in the epididymal tissue at 250 and 500 mg/kg DBP. They showed that DBP exposure altered the structure and function of epididymis by inducing oxidative stress. In another study, DEHP led to a significant decrease in GSH/GSSG redox ratio and increase in thiobarbituric acid reactive substances (TBARSs) levels indicating DEHP induced oxidative stress in rat testis [99]. Earlier, Lee et al. [100] induced hyperthyroidism in pubertal male rats by injecting triiodothyronine (10 μg/kg) intraperitoneally with a simultaneous administration of DBP (750 mg/kg) to normal or hyperthyroid rats. Serum T_3 levels were significantly higher while the serum thyroid stimulating hormone levels were markedly lower in the hyperthyroid rats. They found that hyperthyroidism can cause a change in the expression level of PPARγ in testes and may increase the levels of oxidative damage induced by the metabolic activation of DBP. Sperm motility and the anti-oxidative systems get affected by DBP exposure. DBP showed inhibiting effect on SOD activities in the testis, and it was significant in 1000 mg/kg group [101]. In another study by Wang et al. [102], GSHPx activities in the serum and GSH levels in the testis homogenate showed a decreasing tendency with respect to control, but GSHPx activities increased markedly in the testis, after 2-week DBP exposure. After 4-week DBP exposure, alkaline phosphatase (ALP) activities in the serum increased; SDH activities were significantly inhibited in both the serum and the testis homogenate at 1000 mg/kg and GSH contents in the serum were also affected. A study by Kasahara et al. [86] also indicated that administration of DEHP increased ROS generation and decreased testicular GSH and ascorbic acid leading to apoptosis of spermatocytes. Later, So et al. [103], also reported that DBP and MBP induced developmental toxicity in rat embryonic limb bud cells which was thought to be through oxidative stress exerted by DBP. Recently, Sedha et al. [104] reviewed that phthalate compounds may also induce oxidative stress in the male reproductive organs i.e. testis and epididymis. Oxidative stress and apoptosis in germ cells or target sertoli cells result in impairment of spermatogenesis. They hamper the Leydig cell function by inducing ROS, thereby decreasing the levels of steroidogenic enzymes.

10. Mechanism of phthalate toxicity

Definite understanding of mechanism of toxicity of phthalate is not fully known. However, several pathways are involved to induce toxicity by the phthalates. Some of the phthalates work as endocrine disruptors and are thought to be anti-androgenic. Under in vitro condition, phthalates are not androgen receptor (AR) antagonists directly at concentrations of up to 10 μM, so phthalates and their metabolites do not bind to the AR [91]. Endocrine disruptors (EDs) also act by altering the function of the hypothalamus-pituitary-gonadal (HPG) axis [105,106]. Based upon the mechanism of toxicity [107], the secretion of gonadotrophin releasing hormone (GnRH) is controlled by hypothalamic kisspeptin-1 (KiSS-1) and its G protein-coupled receptor (GPR54) that regulates the anterior pituitary hormones (luteinizing [LH] and follicle stimulating hormone [FSH]) and testicular hormones (testosterone, activin, and inhibin B). As shown in the Fig. 4, EDs alter four functions. 1) Eds modulate KiSS-1/GPR54 and HPG axis. 2) EDs dysregulate pituitary FSH/LH secretion causing reduction in the expression of testicular LH and FSH receptors. 3) EDs interfere with steroidogenic acute regulatory protein (StAR), P450scc, 3β-HSD, and 17β-HSD involved in steroidogenesis. The altered serum levels of the steroid hormones may cause subsequent reproductive dysfunction by interfering with the feedback regulatory mechanisms of the HPG axis. This also stimulates aromatase enzyme leading to estradiol production and reduction in testosterone production. 4) Oxidative stress by EDs causes disruption of sertoli-sertoli and sertoli-germ cell interaction hampering spermatogenesis.

One possible mode of action of phthalates is that phthalate metabolites bind to peroxisome proliferators activated receptor (PPAR) [108]. The PPAR family consists of PPARα, PPARβ, and PPARγ receptors [109]. Rat fetal leydig cells (FLCs) express PPARα and PPARγ receptors [110]. Phthalates are known to activate the actions of PPAR receptors [111–113]. Latini et al. [114] proposed that impairment of reproductive development and function in both genders by phthalates relates to abnormal steroid biosynthesis and metabolism and seems to be at least in part mediated by the activation of PPAR signalling pathway as shown in Fig. 4. Another signaling pathway in leydig cells which might be affected by phthalates is the aryl hydrocarbon receptor (AHR) that belongs to transcription factor family. In fact, foetal testes from animals

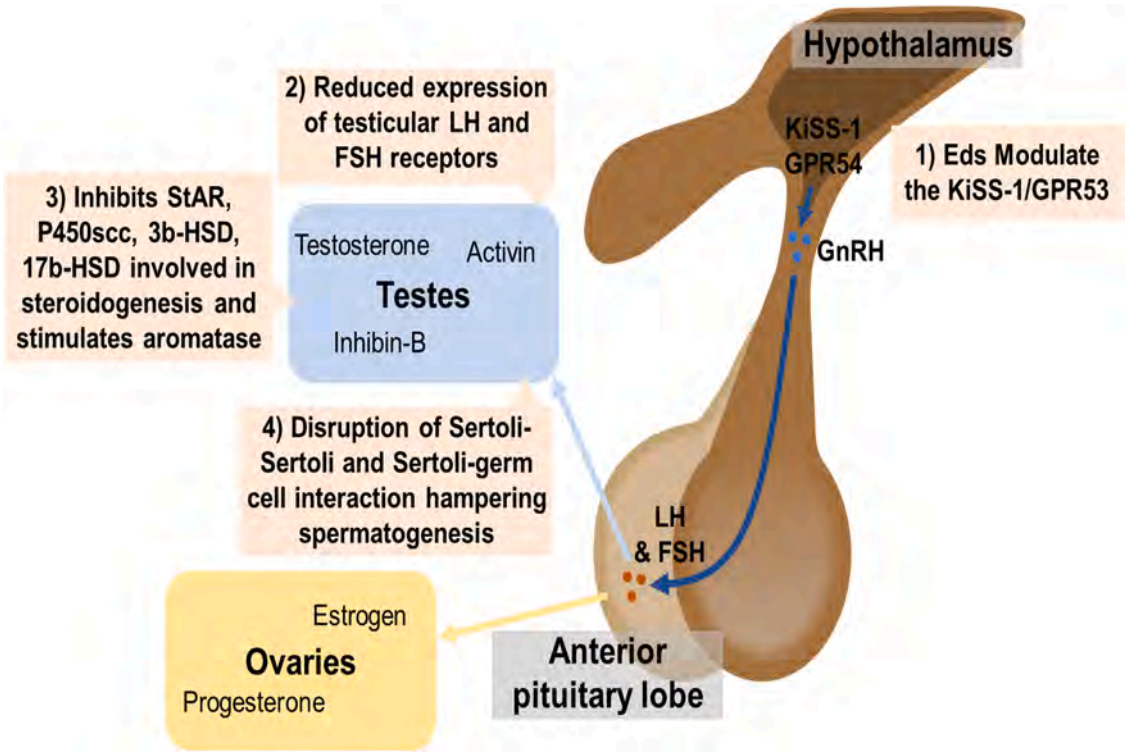


Fig. 4. Multiple effects of phthalate on testicular signaling and spermatogenesis [107].

exposed to phthalates have high expression levels of aryl hydrocarbon receptor and its downstream gene cytochrome Cyp1b1 [115]. Inhibition of leydig cells function may perturb testosterone and Insl-3 synthesis resulting in disturbances in the reproductive tract of male development while interference with sertoli cells may result in failure to proliferate with subsequent depleted germ cells [11]. Generally, it is thought that DBP and DEHP cause leydig cell aggregation and inhibit the production of testosterone as well as Insl-3 while their monoesters act as agonists of PPARα and PPARγ [116]. Thus, several pathways are involved in reproductive toxicity of phthalates.

Crain et al. [117] reviewed the data on the possible role of endocrine-disruptors on female reproductive disorders and mentioned that endocrine disruptors contribute to numerous human female reproductive disorders, emphasizing the sensitivity of early life-stage exposures. Caserta et al. [118] also concluded that for prevention and risk-communication strategies, detailed appraisal of compounds specifically related to adverse reproductive outcomes are very important. In addition to research needs, the current evidence is enough to prompt precautionary actions of exposure to these phthalates to protect reproductive health. Recently, Kumar [119] reported that, in general, working women have a higher risk of undesirable reproductive outcomes and need to educate the child bearing women to avoid exposure to suspected reproductive toxicants. Both men and women should be protected from exposure to reproductive toxicants as sound reproductive health of both is necessary for healthy outcome.

11. Future directions and conclusions

The available studies indicate that phthalates (especially the transitional phthalates) interfere with the normal spermatogenesis leading to testicular atrophy, oxidative stress and DNA damage. They also disrupt the steroidogenic pathways leading to reduced testosterone synthesis and Insl-3 production by the fetal leydig cells which is likely to cause cryptorchidism. The data available indicate the need for the detailed experimental studies on various phthalate compounds on female reproduction, as very sensitive issues of reproductive outcome are involved with the exposure to these chemicals during pregnancy and development. Based upon the reported reproductive effects of phthalates in female even though data are scanty, more robust epidemiological studies are required and preventive steps must be taken to reduce the exposure to these compounds. Owing to this, alternative to phthalates may be developed which do not produce toxicity or uses of phthalates be rationalized.

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References

[1] I.H.S. Markit , Plasticizers: Chemical Economics Handbook, May , 2018. https://ihsmarkit.com/products/plasticizers-chemical-economics-handbook.html.
[2] H. Bahadar, F. Maqbool, A. Mohammad, Consumption of phthalates coated pharmaceutical tablets: an unnoticed threat, Int. J. Pharmacol. 10 (2014) 78–81. https://scialert.net/abstract/?doi=ijp.2014.78.81.
[3] T. Schettler, Human exposure to phthalates via consumer products, Int. J. Androl. 290 (2006) 134–139, https://doi.org/10.1111/j.1365-2605.2005.00567.x.
[4] C.E. Talsness, A.J.M. Andrade, S.N. Kuriyama, J.A. Taylor, F.S. VomSaal, Components of plastic: experimental studies in animals and relevance for human health, Philos. Trans. R. Soc. Lond. B Biol. Sci. 364 (2009) 2079–2096, https://doi.org/10.1098/rstb.2008.0281.
[5] R. Hauser, S. Duty, L. Godfrey-Bailey, A.M. Calafat, Medications as a source of human exposure to phthalates, Environ. Health Perspect. 112 (2004) 751–753, https://doi.org/10.1289/ehp.6804.
[6] FDA- Food and Drug Administration , Inactive ingredient search for approved drug products: Frequently asked questions, 2010. http://www.fda.gov/Drugs/InformationOnDrugs/ucm080123.htm.
[7] S. Saeidnia, M. Abdollahi, Are medicinal plants polluted with phthalates? DARU J. Pharm. Sci. 21 (2013) 43, https://doi.org/10.1186/2008-2231-21-43.

[8] K.T. Bogen, K. Boekelheide, M.L. Cunningham, B.A. Jackson, J.M. Peters, J. K. Reddy, L. Zeise, Report to the US consumer product safety commission by the chronic hazard advisory panel on di-isononyl phthalate, Bethesda (2001).
[9] NICNAS. Existing chemical hazards assessment report. Di-isoundecyl phthalate , 2008. https://www.pharosproject.net/uploads/files/sources/1828/2918024048 6f1a826cdee842031262757ba167bf.pdf.
[10] Phthalate Esters Panel HPV Testing Group, High production volume (HPV) chemical challenge programme test plan for the phthalate esters category, 10 December, 2001. Prepared by: ExxonMobil Biomedical Sciences, Inc. for the Phthalate Esters Panel HPV Testing Group of the American Chemistry Council. http://www.epa.gov/hpv/pubs/summaries/benzene/c13467tc.htm.
[11] Phthalate Hazard Compendium, A summary of physicochemical and human health hazard data for 24 ortho-phthalate chemicals. Existing Chemical Hazard Assessment Report. Phthalate Hazard Compendium, June 2008. National Industrial Chemicals Notification and Assessment Scheme, Australia. https ://www.nicnas.gov.au/search?query=84-74-2&collection=nicnas-meta.
[12] NTP-CERHR, Monograph on the potential human reproductive and developmental effects of di-isononyl phthalate (DINP), 2003a. NIH Publication No. 03–4484.
[13] L.E. Gray Jr, J. Ostby, J. Furr, M. Price, D.N.R. Veeramachaneni, L. Parks, Perinatal exposure to the phthalates DEHP, BBP and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat, Toxicol. Sci. 58 (2000) 350–365, https://doi.org/10.1093/toxsci/58.2.350.
[14] P.M.D. Foster, L.V. Thomas, M.W. Cook, S.D. Gangolli, Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat, Toxicol. Appl. Pharmacol. 54 (3) (1980) 392–398, https://doi.org/10.1016/0041-008X(80)90165-9.
[15] K. Howdeshell, V. Wilson, J. Furr, C. Lambright, C. Rider, C. Blystone, A. Hotchkiss, L.E. Gray, A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rats in a cumulative, dose additive manner, Toxicol. Sci. 105 (2008) 153–165, https://doi.org/10.1093/toxsci/kfn077.
[16] NTP-CERHR , Monograph on the potential human reproductive and developmental effects of di-hexyl phthalate (DHP), 2003b. NIH Publication No. 03–4489.
[17] J. Hellwig, H. Freudenberger, R. Jackh, Differential prenatal toxicity of branched phthalate esters in rats, Food Chem. Toxicol. 35 (5) (1997) 501–512, https://doi.org/10.1016/s0278-6915(97)00008-2.
[18] M.R. Moore, Oncogenicity study in mice with di(isononyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Covance Laboratories 144 Inc., Vienna, VA 22182. For Aristech Chemical Corporation, Pittsburgh, PA 15230. January 29, Covance (1998), 2598–105.
[19] SCENIHR 2008 SCENIHR opinion on the Safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk, European Commission, 2008.
[20] NTP-CERHR , Monograph on the potential human reproductive and developmental effects of di-octyl phthalate (DOP), NIH Publication No. 03–4488, 2003.
[21] B.R. Hannas, C.S. Lambright, J. Furr, K.L. Howdeshell, V.S. Wilson, L.E. Gray Jr, Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate and diisononyl phthalate, Toxicol. Sci. 123 (1) (2011) 206–216, https://doi.org/10.1093/toxsci/kfr146.
[22] R. Ahmad, A.K. Gautam, Y. Verma, S. Sedha, S. Kumar, Effects of in utero di-butyl phthalate and butyl benzyl phthalate exposure on offspring development and male reproduction of rat, Environ. Sci. Pollut. Res. 21 (4) (2014) 3156–3165, https://doi.org/10.1007/s11356-013-2281-x.
[23] J. Borch, O. Ladefoged, U. Hass, A.M. Vinggaard, Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, pre-pubertal and adult male rats, Reprod. Toxicol. 18 (1) (2004) 53–61, https://doi.org/10.1016/j.reprotox.2003.10.011.
[24] R.A. Clewell, A. Thomas, G. Willson, D.M. Creasy, M.E. Andersen, A dose response study to assess effects after dietary administration of di-isononyl phthalate (DINP) in gestation and lactation on male rat sexual development, Reprod. Toxicol. 35 (2013) 70–80, https://doi.org/10.1016/j.reprotox.2012.07.008.
[25] S.J. Waterman, J.L. Ambroso, L.H. Keller, G.W. Trimmer, A.I. Nikiforov, S. B. Harris, Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats, Reprod. Toxicol. 13 (2) (1999) 131–136, https://doi.org/10.1016/s0890-6238(99)00002-7.
[26] N. Masutomi, M. Shibutani, H. Takagi, C. Uneyama, N. Takahashi, M. Hirose, Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life, Toxicology 192 (2003) 149–170, https://doi.org/10.1016/s0300-483x(03)00269-5.
[27] J. Boberg, S. Christiansen, M. Axelstad, T.S. Kledal, A.M. Vinggaard, M. Dalgaard, C. Nellemann, U. Hass, Reproductive and behavioural effects of diisononylphthalate (DINP) in perinatally exposed rats, Reprod. Toxicol. 31 (2) (2011) 200–209, https://doi.org/10.1016/j.reprotox.2010.11.001.
[28] R.A. Clewell, M. Sochaski, K. Edwards, D.M. Creasy, G. Willson, M.E. Andersen, Disposition of diisononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats, Reprod. Toxicol. 35 (2013) 56–69, https://doi.org/10.1016/j.reprotox.2012.07.001.
[29] B.D. Hardin, R.L. Schuler, J.R. Burg, G.M. Booth, K.P. Hazelden, K. M. MackKenzie, V.J. Piccirillo, K.N. Smith, Evaluation of 60 chemicals in a preliminary developmental toxicity test, Teratog. Carcinog. Mutagen. 7 (1987) 29–48, https://doi.org/10.1002/tcm.1770070106.
[30] J. Borch, M. Axelstad, A.M. Vinggaard, M. Dalgaard, Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis, Toxicol. Lett. 163 (2006) 183–190, https://doi.org/10.1016/j.toxlet.2005.10.020.
[31] J. Boberg, S. Metzdorff, R. Wortziger, M. Axelstad, L. Brokken, A.M. Vinggaard, M. Dalgaard, C. Nellemann, Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats, Toxicology 250 (2008) 75–81, https://doi.org/10.1016/j.tox.2008.05.020.
[32] A.M. Saillenfait, J.P. Sabate, F. Gallissot, Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butylphthalate administration by gavage to rats, Toxicol. Lett. 165 (2006) 39–46, https://doi.org/10.1016/j.toxlet.2006.01.013.
[33] A.M. Saillenfait, J.P. Sabate, F. Gallissot, Di-isobutyl phthalate impairs the androgen-dependent reproductive development of the male rat, Reprod. Toxicol. 26 (2008) 107–115, https://doi.org/10.1016/j.reprotox.2008.07.006.
[34] S.M. Duty, M.J. Silva, D.B. Barr, J.W. Brock, L. Ryan, Z. Chen, R.F. Herrick, D. C. Christiani, R. Hauser, 2003 Phthalate exposure and human semen parameters, Epidemiology 14 (3) (2003) 269–277. PMID: 12859026.
[35] R. Hauser, J.D. Meeker, S. Duty, M.J. Silva, A.M. Calafat, Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites, Epidemiology 17 (6) (2006) 682–691, https://doi.org/10.1097/01.ede.0000235996.89953.d7.
[36] Y. Pan, J. Jing, F. Dong, Q. Yao, W. Zhang, H. Zhang, B. Yao, J. Dai, Association between phthalate metabolites and biomarkers of reproductive function in 1066 Chinese men of reproductive age, J. Hazard. Mater. 300 (2015) 729–736, https://doi.org/10.1016/j.jhazmat.2015.08.011.
[37] Y. Wang, Q. Zeng, Y. Sun, P. Yang, P. Wang, J. Li, Z. Huang, L. You, Y.H. Huang, C. Wang, Y.F. Li, W.Q. Lu, Semen phthalate metabolites, semen quality parameters and serum reproductive hormones: a cross-sectional study in China, Environ. Poll. 211 (2016) 173–182, https://doi.org/10.1016/j.envpol.2015.12.052.
[38] B.B. Hoyer, V. Lenters, A. Giwercman, B.A.G. Jonsson, G. Toft, K.S. Hougaard, J. P.E. Bonde, I.O. Specht, Impact of di-2-ethylhexyl phthalate metabolites on male reproductive function: a systematic review of human evidence, Curr. Environ. Health Rep. 5 (1) (2018) 20–33, https://doi.org/10.1007/s40572-018-0174-3.
[39] S.H. Swan, K.M. Main, F. Liu, S.L. Stewart, R.L. Kruse, A.M. Calafat, C.S. Mao, J. B. Redmon, C.L. Ternand, S. Sullivan, J.L. Teague, Decrease in anogenital distance among male infants with prenatal phthalate exposure, Environ. Health Perspect. 113 (2005) 1056–1061, https://doi.org/10.1289/ehp.8100.
[40] J.A. McLachlan, E. Simpson, M. Martin, Endocrine disrupters and female reproductive health, Best. Pract. Res. Clin. Endocrinol. Metab. 20 (2006) 63–75, https://doi.org/10.1016/j.beem.2005.09.009.
[41] A.J. Martino-Andrade, I. Chahoud, Reproductive toxicity of phthalates esters, Mol. Nutr. Food Res. 54 (1) (2010) 148–157, https://doi.org/10.1002/mnfr.200800312.
[42] L.E. Gray Jr, J. Laskey, J. Ostby, Chronic di-n-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female long evans hooded rats, Toxicol. Sci. 93 (2006) 189–195, https://doi.org/10.1093/toxsci/kfl035.
[43] B.J. Davis, R.R. Maronpot, J.J. Heindel, Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats, Toxicol. Appl. Pharmacol. 128 (1994) 216–223, https://doi.org/10.1006/taap.1994.1200.
[44] S.T. Lovekamp, B.J. Davis, Mechanisms of phthalate ester toxicity in the female reproductive system, Environ. Health Perspect. 11 (2003) 139–145, https://doi.org/10.1289/ehp.5658.
[45] M. Mingyue, T. Kondo, S. Ban, T. Umemura, N. Kurahashi, M. Takeda, R. Kishi, Exposure of pre-pubertal female rats to inhale di(2-ethylhexyl)phthalate affects the onset of puberty and post-pubertal reproductive functions, Toxicol. Sci. 93 (1) (2006) 164–171, https://doi.org/10.1093/toxsci/kfl036.
[46] R. Ahmad, Y. Verma, A.K. Gautam, S. Kumar, Assessment of estrogenic potential of di-n-butyl phthalate and butyl benzyl phthalate in vivo, Toxicol. Ind. Health 31 (12) (2015) 1296–1303, https://doi.org/10.1177/0748233713491803.
[47] S. Sedha, A.K. Gautam, Y. Verma, R. Ahmad, S. Kumar, Determination of in vivo estrogenic potential of di-isobutyl phthalate (DIBP) and di-isononyl phthalate (DINP) in rats, Environ. Sci. Poll. Res. 22 (2015) 18197–18202, https://doi.org/10.1007/s11356-015-5021-6.
[48] P. Pocar, N. Fiandanese, C. Secchi, A. Berrini, B. Fischer, J.S. Schmidt, K. Schaedlich, V. Borromeo, 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 153 (2012) 937–948, https://doi.org/10.1210/en.2011-1450.
[49] R. Moral, P.J. Santucci, R. Wang, I.H. Russo, C.A. Lamartiniere, J. Russo, 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 10 (1) (2011) 5, https://doi.org/10.1186/1476-069X-10-5.
[50] M.T. Guerra, W.R. Scarano, F.C. de Toledo, J.A. Franci, W. Kempinas, Reproductive development and function of female rats exposed to di-eta-butyl-phthalate (DBP) in utero and during lactation, Reprod. Toxicol. 29 (1) (2010) 99–105, https://doi.org/10.1016/j.reprotox.2009.10.005.
[51] K.Y. Lee, M. Shibutani, H. Takagi, N. Kato, S. Takigami, C. Uneyama, M. Hirose, 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 203 (2004) 221–238, https://doi.org/10.1016/j.tox.2004.06.013.
[52] S.W. Grande, J.M. Andrade Anderson, C.E. Talsness, K. Grote, I. Chahoud, A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)

phthalate: effects on female rat reproductive development, Toxicol. Sci. 91 (2006) 247–254, https://doi.org/10.1093/toxsci/kfj128.
[53] M.S. Piepenbrink, I. Hussain, J.A. Marsh, R.R. Dietert, Developmental Immunotoxicology of di-(2-ethylhexyl) phthalate (DEHP). Age-based assessment in the female rat, J. Immunotoxicol. 2 (2005) 21–31, https://doi.org/10.1080/15363750490429435.
[54] Y. Ding, Y. Gao, R. Shi, Y.J. Zhou, Y. Tian, Effects of in utero exposure to di(2-ethylhexyl) phthalate on sexual development in female offspring, Chinese J. Prev. Med. 44 (2) (2010) 150–153. PMID: 20388337.
[55] M.J. Silva, J.A. Reidy, A.R. Herbert, J.L. Preau Jr., L.L. Needham, A.M. Calafat, Detection of phthalate metabolites in human amniotic fluid, Bull. Environ. Contam. Toxicol. 72 (2004) 1226–1231, https://doi.org/10.1007/s00128-004-0374-4.
[56] M. Wittassek, J. Angerer, G.M. Kolossa, S.D. Schafer, W. Klockenbusch, L. Dobler, K. Gunsel Andreas, A. Muller, G.A. Wiesmuller, Fetal exposure to phthalates – a pilot study, Int. J. Hyg. Environ. Health 212 (2009) 492–498, https://doi.org/10.1016/j.ijheh.2009.04.001.
[57] Y. Zhang, L. Lin, Y. Cao, B. Chen, L. Zheng, S. Ge Ren, Phthalate levels and low birth weight: a nested case-control study of Chinese new-borns, J. Pediatr. 155 (2009) 500–504, https://doi.org/10.1016/j.jpeds.2009.04.007.
[58] G. Toft, B.A. Jonsson, C.H. Lindh, T.K. Jensen, N.H. Hjollund, A. Vested, J. P. Bonde, Association between pregnancy loss and urinary phthalate levels around the time of conception, Environ. Health Perspect. 120 (2012) 458–463, https://doi.org/10.1289/ehp.1103552.
[59] S.H. Kim, S. Chun, J.Y. Jang, D.H. Chae, C.H. Kim, B.M. Kang, Increased plasma levels of phthalate esters in women with advanced-stage endometriosis: a prospective case-control study, Fertil. Steril. 95 (2011) 357–359, https://doi.org/10.1016/j.fertnstert.2010.07.1059.
[60] B.S. Reddy, R. Rozati, S. Reddy, S. Kodampur, P. Reddy, R. Reddy, High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study, Fertil. Steril. 85 (2006) 775–779, https://doi.org/10.1016/j.fertnstert.2005.08.037.
[61] J. Weuve, R. Hauser, A.M. Calafat, S.A. Missmer, L.A. Wise, Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999–2004, Environ. Health Perspect. 118 (2010) 825–832, https://doi.org/10.1289/ehp.0901543.
[62] H. Itoh, M. Iwasaki, T. Hanaoka, H. Sasaki, T. Tanaka, S. Tsuhane, Urinary phthalate monoesters and endometriosis in infertile Japanese women, Sci. Total Environ. 408 (2009) 37–42, https://doi.org/10.1007/BF02898033.
[63] C. Xu, H. Lini, Y. Zhao, Y. Zhang, Determination of serum levels of three phthalates esters in patients with polycystic ovary syndrome, Sci. Res. Essays 6 (5) (2011) 1057–1062, https://doi.org/10.5897/SRE10.918.
[64] A. Burdorf, T. Brand, V.W. Jaddoe, A. Hofman, J.P. Mackenbach, E.A.P. Steegers, The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: the generation R study, Occup. Environ. Med. 68 (2011) 197–204, https://doi.org/10.1136/oem.2009.046516.
[65] I. Colon, D. Caro, C.J. Bourdony, O. Rosario, Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development, Environ. Health Perspect. 108 (2000) 895–900, https://doi.org/10.1289/ehp.108-2556932.
[66] Y.Y. Chou, P.C. Huang, C.C. Lee, M.H. Wu, S.J. Lin, Phthalate exposure in girls during early puberty, J. Pediatr. Endocrinol. Metab. 22 (1) (2009) 69–77, https://doi.org/10.1515/jpem.2009.22.1.69.
[67] E. Durmaz, P. Erkekoglu, A. Asci, S. Akvcurin, I. Bircan, B. Kocer-Gumusel, Urinary phthalate metabolite concentrations in girls with premature thelarche, Environ. Toxicol. Pharmacol. 59 (2018) 172–181, https://doi.org/10.1016/j.etap.2018.03.010.
[68] J.P. Lomenick, A.M. Calafat, M.S. Melguizo Castro, R. Mier, P. Stenger, M. B. Foster, K.A. Wintergerst, Phthalate exposure and precocious puberty in females, J. Pediatr 156 (2) (2010) 221–225, https://doi.org/10.1016/j.jpeds.2009.09.047.
[69] R.H. McKee, Phthalate exposure and early thelarche, Environ. Health Perspect. 112 (2004) 541–543, https://doi.org/10.1289/ehp.112-a541b.
[70] J. Jurewicz, W. Hanke, Exposure to phthalates: reproductive outcome and children health. A review of epidemiological studies!, Int. J. Occup. Med. Environ. Health 24 (2011) 115–141, https://doi.org/10.2478/s13382-011-0022-2. Abstract.
[71] M.R. Parkhie, M. Webb, M.A. Norcross, Dimethoxy ethyl phthalate: embryopathy, teratogenicity, fetal metabolism and the role of zinc in the rat, Environ. Health Perspect. 45 (1982) 89–97, https://doi.org/10.1289/ehp.824589.
[72] K. Shiota, M.J. Chou, H. Nishimura, Embryotoxic effects of di-2-ethylhexyl phthalate (DEHP) in mice, Environ. Res. 22 (1980) 245–253, https://doi.org/10.1016/0013-9351(80)90136-x.
[73] E. Makoto, E. Miyawaki, K. Kawashima, Reproductive effects of butyl benzyl phthalate in pregnant and pseudopregnant rats, Reprod. Toxicol. 12 (1998) 127–132, https://doi.org/10.1016/s0890-6238(97)00127-5.
[74] E. Makoto, I. Takafumi, K. Hironoshin, Teratogenic evaluation of butyl benzyl phthalate in rats by gastric intubation, Toxicol. Lett. 61 (1992) 1–7, https://doi.org/10.1016/0378-4274(92)90057-Q.
[75] E. Makoto, I. Takafumi, K. Hironoshin, Teratogenic phase specificity of butyl benzyl phthalate in rats, Toxicology 79 (1993) 11–19, https://doi.org/10.1016/0300-483X(93)90202-4.
[76] P.G. Wells, G.P. McCallum, C.S. Chen, J.T. Henderson, C.J. Lee, J. Perstin, T. J. Preston, M.J. Wiley, A.W. Wong, Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer, Toxicol. Sci. 108 (1) (2009) 4–18, https://doi.org/10.1093/toxsci/kfn263.
[77] P.P. Mathur, S.C. Cruz, The effect of environmental contaminants on testicular function, Asian J. Androl. 13 (2011) 585–591, https://doi.org/10.1038/aja.2011.40.
[78] P.P. Mathur, B. Saradha, S. Vaithinathan, Impact of environmental toxicants on testicular function, Immun. Endocr. Metab. Agents Med. Chem. 8 (2008) 79–90, https://doi.org/10.2174/187152208783790705.
[79] E.H. Ruder, T.J. Hartman, J. Blumberg, M.B. Goldman, Oxidative stress and antioxidants: exposure and impact on female fertility, Hum. Reprod. Update 14 (4) (2008) 345–357, https://doi.org/10.1093/humupd/dmn011.
[80] S. Murarka, A.K. Gautam, Y. Verma, V. Shivgotra, H. Doshi, S. Kumar, Association between sperm quality, oxidative stress and seminal antioxidant activity, Clin. Biochem. 44 (4) (2011) 319–324, https://doi.org/10.1016/j.clinbiochem.2010.11.009.
[81] R.J. Aitken, S.D. Roman, Antioxidant systems and oxidative stress in the testes, Oxid. Med. Cell. Longev. 1 (1) (2008) 15–24, https://doi.org/10.4161/oxim.1.1.6843.
[82] Y. Ito, O. Yamanoshita, N. Asaeda, Y. Tagawa, C.H. Lee, T. Aoyama, G. Ichihara, K. Furuhashi, M. Kamijima, F.J. Gonzalez, T. Nakajima, Di (2-ethylhexyl) phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway, J. Occup. Health 49 (2007) 172–182, https://doi.org/10.1539/joh.49.172.
[83] D. Zhou, H. Wang, J. Zhang, Di-n-butyl phthalate (DBP) exposure induces oxidative stress in epididymis of adult rats, Toxicol. Ind. Health 27 (1) (2011) 65–71, https://doi.org/10.1177/0748233710381895.
[84] A. Wellejus, M. Dalgaard, S. Loft, Oxidative DNA damage in male Wistar rats exposed to di-n-butyl phthalate, J. Toxicol. Environ. Health A 65 (2002) 813–824, https://doi.org/10.1080/00984100290071126.
[85] K.W. Seo, K.B. Kim, Y.J. Kim, J.Y. Choi, K.T. Lee, K.S. Choi, Comparison of oxidative stress, Chem. Toxicol. 42 (1) (2004) 107–114. https://search.proquest.com/docview/71565761?accountid=171501.
[86] E. Kasahara, E.F. Sato, M. Miyoshi, R. Konaka, K. Hiramoto, J. Sasaki, M. Tokuda, Y. Nakano, M. Inoue, Role of oxidative stress in germ cell apoptosis induced by di (2-ethylhexyl) phthalate, Biochem. J. 365 (2002) 849–856, https://doi.org/10.1042/BJ20020254.
[87] V.S. Wilson, C. Lambright, J. Furr, J. Ostby, C. Wood, G. Held, L.E. Gray, Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis, Toxicol. Lett. 146 (2004) 207–215, https://doi.org/10.1016/j.toxlet.2003.09.012.
[88] V. Peltola, I. Huhtaniemi, T. Metsa-Ketela, M. Ahotupa, Induction of lipid peroxidation during steroidogenesis in the rat testis, Endocrinology 137 (1996) 105–112, https://doi.org/10.1210/en.137.1.105.
[89] K. Liu, K.P. Lehmann, M. Sar, S. Young, K.W. Gaido, Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis, Biol. Reprod. 73 (2005) 180–192, https://doi.org/10.1095/biolreprod.104.039404.
[90] J. Borch, M. Dalgaard, O. Ladefoged, Early testicular effects in rats perinatally exposed to DEHP in combination with DEHA-apoptosis assessment and immunohistochemical studies, Reprod. Toxicol. 19 (2005) 517–525, https://doi.org/10.1016/j.reprotox.2004.11.004.
[91] L.G. Parks, J.S. Ostby, C.R. Lambright, B.D. Abbott, G.R. Klinefelter, N.J. Barlow, L.E. Gray Jr, The plasticizer diethylhexyl phthalate induces malformations by decreasing foetal testosterone synthesis during sexual differentiation in the male rat, Toxicol. Sci. 58 (2000) 339–349, https://doi.org/10.1093/toxsci/58.2.339.
[92] T. Nagao, R. Ohta, H. Marumo, T. Shindo, S. Yoshimura, H. Ono, Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study, Reprod. Toxicol. 14 (2000) 513–532, https://doi.org/10.1016/s0890-6238(00)00105-2.
[93] E. Mylchreest, M. Sar, D.G. Wallace, P.M.D. Foster, Foetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate, Reprod. Toxicol. 16 (1) (2002) 19–28, https://doi.org/10.1016/s0890-6238(01)00201-5.
[94] K.P. Lehmann, S. Phillips, M. Sar, P.M. Foster, K.W. Gaido, Dose-dependent alterations in gene expression and testosterone synthesis in the foetal testes of male rats exposed to di(n-butyl) phthalate, Toxicol. Sci. 81 (2004) 60–68, https://doi.org/10.1093/toxsci/kfh169.
[95] A.J. Kuhl, S.M. Ross, K.W. Gaido, CCAAT/enhancer binding protein {beta}, but not SF-1, modulates the phthalate-induced dysregulation of rat fetal testicular steroidogenesis, Endocrinology 148 (12) (2007) 5851–5864, https://doi.org/10.1210/en.2007-0930.
[96] Y.C. Hong, E.Y. Park, M.S. Park, J.A. Ko, S.Y. Oh, H. Kim, K.H. Lee, J.H. Leem, E. H. Ha, Community level exposure to chemicals and oxidative stress in adult population, Toxicol. Lett. 184 (2) (2009) 139–144, https://doi.org/10.1016/j.toxlet.2008.11.001.
[97] K. Ji, Y.L. Kho, Y. Park, K. Choi, Influence of a five-day vegetarian diet on urinary levels of antibiotics and phthalate metabolites: a pilot study with “temple stay” participants, Environ. Res. 110 (4) (2010) 375–382, https://doi.org/10.1016/j.envres.2010.02.008.
[98] N. Ma, W. Zhang, Y. Feng, H. Xu, Effect of diisobutyl phthalate on antioxidase activity and DNA damage in mice, Wei Sheng Yan Jiu 39 (2010) 549–551.
[99] P. Erkekoglu, B. Giray, W. Rachidi, I. Hininger-Favier, A.M. Roussel, A. Favier, F. Hincal, Effects of di(2-ethylhexyl)phthalate on testicular oxidant/antioxidant status in selenium-deficient and selenium-supplemented rats, Environ. Toxicol. 29 (1) (2014) 98–107, https://doi.org/10.1002/tox.20776.
[100] E. Lee, M.Y. Ahn, H.J. Kim, I.Y. Kim, S.Y. Han, T.S. Kang, J.H. Hong, K.L. Park, B. M. Lee, H.S. Kim, Effect of di(n-butyl) phthalate on testicular oxidative damage

and antioxidant enzymes in hyperthyroid rats, Environ. Toxicol. 22 (3) (2007) 245–255, https://doi.org/10.1002/tox.20259.
[101] Y. Wang, L. Song, Z. Zhu, J. Chen, J. He, R. Liu, X. Wang, Effects of dibutyl phthalate on sperm motility and oxidative stress in rats, Zhonghua Nan Ke Xue 10 (4) (2004) 253–256.
[102] Y. Wang, L. Song, Z. Zhu, J. Chen, J. He, R. Liu, X. Wang, Effect of dibutyl phthalate on the biochemical enzymes and lipid peroxidation in rat testes, Zhonghua Nan Ke Xue 10 (10) (2004) 729–733.
[103] H.K. So, S.K. Soon, K. Oan, H.S. Kyung, J.K. Seung, W.C. Yo, Effects of dibutyl phthalate and monobutyl phthalate on cytotoxicity and differentiation in cultured rat embryonic limb bud cells; protection by antioxidants, J. Toxicol. Environ. Health 65 (2002) 461–472, https://doi.org/10.1080/15287390252808118.
[104] S. Sedha, S. Kumar, S. Shukla, Role of oxidative stress in male reproductive dysfunctions with reference to phthalate compounds, Urol. J. 12 (5) (2015) 2304–2316, https://doi.org/10.22037/uj.v12i5.3009.
[105] M. Tena-Sempere, Kisspeptin/GPR54 system as potential target for endocrine disruption of reproductive development and function, Int. J. Androl. 33 (2010) 360–368, https://doi.org/10.1111/j.1365-2605.2009.01012.x.
[106] M. Bellingham, P.A. Fowler, M.R. Amezaga, S.M. Rhind, C. Cotinot, B. Mandon-Pepin, R.M. Sharp, N.P. Evans, Exposure to a complex cocktail of environmental endocrine-disrupting compounds disturbs the kisspeptin/GPR54 system in ovine hypothalamus and pituitary gland, Environ. Health Perspect. 117 (10) (2009) 1556–1562. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2790510/.
[107] H.Y. Yeung Bonnie, T. Wan Hin, Y.S. Law Alice, K.C. Wong Chris, Endocrine disrupting chemicals. Multiple effects on testicular signaling and spermatogenesis, Spermatogenesis 1 (3) (2011) 231–239, https://doi.org/10.4161/spmg.1.3.18019.
[108] M. Gazouli, Z.X. Yao, N. Boujrad, J.C. Corton, M. Culty, V. Papadopoulos, Effect of peroxisome proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport and steroidogenesis: role of the peroxisome proliferator-activator receptor alpha, Endocrinology 143 (2002) 2571–2583, https://doi.org/10.1210/endo.143.7.8895.
[109] T. Lemberger, B. Desvergne, W. Wahli, Peroxisome proliferator-activated receptors: a nuclear receptor-signaling pathway in lipid physiology, Ann. Rev. Cell. Dev. Biol. 12 (1996) 335–363, https://doi.org/10.1146/annurev.cellbio.12.1.335.
[110] R. Schultz, W. Yan, J. Toppari, A. Alfred Volkl, J.A. Gustafsson, M. Pelto-huikko, Expression of peroxisome proliferator-activated receptor alpha messenger ribonucleic acid and protein in human and rat testis, Endocrinology 140 (1999) 2968–2975, https://doi.org/10.1210/endo.140.7.6858.
[111] J.C. Corton, P.J. Lapinskas, Peroxisome proliferator-activated receptors: mediators of phthalate ester induced effects in the male reproductive tract, Toxicol. Sci. 83 (2005) 4–17, https://doi.org/10.1093/toxsci/kfi011.
[112] C.H. Hurst, D.J. Waxman, Activation of PPARα and PPARγ by environmental phthalate monoesters, Toxicol. Sci. 74 (2003) 297–308, https://doi.org/10.1093/toxsci/kfg145.
[113] A. Lampen, S. Zimnick, H. Nau, Teratogenic phthalate esters and metabolites activate the nuclear receptors PPARs and induce differentiation of F9 cells, Toxicol. Appl. Pharmacol. 188 (2003) 14–23, https://doi.org/10.1016/s0041-008x(03)00014-0.
[114] G. Latini, E. Scoditti, A. Verrotti, C.D. Felice, M. Massaro, Peroxisome proliferator-activated receptors as mediators of phthalate-induced effects in the male and female reproductive tract: epidemiological and experimental evidence, PPAR Res. 2008 (2008) 1–13, https://doi.org/10.1155/2008/359267.
[115] S.T. Lovekamp, A.M. Jelten, B.J. Davis, Dual activation of PPARα and PPARγ by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells, Mol. Cell Endocrinol. 201 (2003) 133–141, https://doi.org/10.1016/s0303-7207(02)00423-9.
[116] G.X. Hu, Q.Q. Lian, R.S. Ge, D.O. Hardy, X.K. Li, Phthalate induced testicular dysgenesis syndrome: leydig cell influence, Trends Endocrinol. Metab. 20 (2009) 139–145, https://doi.org/10.1016/j.tem.2008.12.001.
[117] D.A. Crain, S.J. Janssen, T.M. Edwards, J. Heindel, S.M. Ho, P. Hunt, T. Iguchi, A. Juul, J.A. McLachlan, J. Schwartz, N. Skakkebaek, A.M. Soto, S. Swan, C. Walker, T.K. Woodruff, T.J. Woodruff, L.C. Guidice, L.J. Guilette Jr., Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing, Fertil. Steril. 90 (2008) 911–940, https://doi.org/10.1016/j.fertnstert.2008.08.067.
[118] D. Caserta, A. Mantovani, R. Marci, A. Fazi, F. Ciardo, C. La Rocca, F. Maranghi, M. Moscarini, Environment and women's reproductive health, Human. Reprod. Update 17 (2011) 418–433, https://doi.org/10.1093/humupd/dmq061.
[119] S. Kumar, Occupational, environmental and lifestyle factors associated with spontaneous abortion, Reprod. Sci. 18 (10) (2011) 915–930, https://doi.org/10.1177/1933719111413298.
[120] Y. Xue, Yi Qian-hui, Z. Qiao-Qiao, M. Wen, C. Zhang, H. Zhen, H. Xiao, X. Jing, X. Li, L. Di, Comparison of estrogenic activity of phthalate esters in vitro, Environ. Sci. Technol. 34 (3) (2011) 1–5. HERO ID: 1249555.
[121] Q. Jin, Y. Li, Z. Sun, Estrogenic activities of di-2-ethylhexyl phthalate, Fornt. Med. China 2 (2008) 303–308, https://doi.org/10.1007/s11684-008-0058-2.
[122] Y.J. Kim, J.C. Riu, Evaluation of estrogenic effects of phthalate analogues using in vitro and in vivo screening assays, Mol. Cell. Toxicol. 2 (2006) 106–113. http://www.tox.or.kr/toxsoc/media/papers/2009/05/08/225_.pdf.
[123] Y. Hashimoto, M. Kawaquchi, K. Miyazaki, M. Nakamura, Estrogenic activity of tissue conditioners in vitro, Dent. Mater. 19 (2003) 341–346, https://doi.org/10.1016/s0109-5641(02)00064-7.
[124] O. Tomoko, S. Toshinari, Y. Yoshiko, K. Kazutaka, K. Itsu, Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by mcf-7 cell proliferation assay in vitro, Biol. Pharm. Bull. 26 (2003) 1219–1224, https://doi.org/10.1248/bpb.26.1219.
[125] T.R. Zacharewski, M.D. Meek, J.H. Clemons, Z.F. Wu, M.R. Fielden, J. B. Matthews, Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters, Toxicol. Sci. 46 (1998) 282–293, https://doi.org/10.1006/toxs.1998.2505.
[126] C.A. Harris, P. Henttu, M.G. Parker, J.P. Sumpter, The estrogenic activity of phthalate esters in vitro, Environ. Health Perspect. 105 (8) (1997) 802–811, https://doi.org/10.1289/ehp.97105802.