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Phthalates Exposure and Endocrinal Effects: An Epidemiological Review

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ABSTRACT

Phthalates are ubiquitous endocrine disruptors which may cause potential health effects in general populations. We explored 3 scientific databases (PubMed, Medline, and ScienceDirect) to search epidemiological studies focused on phthalates exposure and the important health effects including endocrine, hormonal regulation of thyroid and steroid, reproductive effects, pregnancy, precocious puberty, obesity, and infertility in human. Some studies demonstrated negative association between phthalate levels and sex steroid hormones (testosterone, LH), thyroid hormones (thyroxine), sperm quality, anogenital distance, gestational age and neurodevelopment (cognitive function and intelligence quotient), although most of them have some limitations. A few studies showed positive association between phthalate levels and precocious puberty, pregnancy loss, obesity, leiomyoma, although results of some studies were not consistent. In summary, phthalates may affect reproductive and sex hormones, thyroid function and development. Although large-scale cohort studies are needed to clarify the association, it is necessary to reduce the phthalates exposure in pregnant women and young children to prevent unexpected consequences of reproductive and development effects in the offspring.

Key words: phthalate, endocrine, reproduction, thyroid function, neurodevelopment, infertility

INTRODUCTION

Phthalates are considered endocrine disrupting chemicals in many aspects, including estrogenic, anti-androgenic, and anti-thyroid activites[1-3]. They are suspected to interfere with the production, activation and excretion of natural hormones in human, especially in reproductive system.

Phthalates are added to plastics to make them soft and flexible, to cosmetics as a vehicle for fragrances, and to many other daily products, including building materials, children's toys, and medical devices[4]. Recent reports have shown that phthalates are widely added in considerable amounts (up to 5%) to cosmetics and personal care products, which raise the levels of urinary phthalates metabolites rapidly when these products are used daily[5,6], particularly in women. The potential consequences of human exposure to phthalates have raised concerns in the general population and have been studied in susceptible subjects, such as pregnant women, infants, and children[7-9].

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Phthalates are estrogenic and anti-androgenic endocrine disruptors that may prolong menstrual cycles and increase the proportion of premature menopause in animal models[1,2]. Toxicological evidence has shown that some phthalates, such as butyl benzyl phthalate (BBzP; BBP), di-n-butyl phthalate (DnBP;DBP), and di-(2-ethylhexyl) phthalate (DEHP), may alter or mimic estradiol (E_2) *in vivo* and *in vitro*[10-12]. However, whether phthalate exposure results in adverse effects on human reproductive system remains largely unknown.

Toxicological evidence showed that the fetus is exposed to some phthalates, such as DBP, diethyl phthalate (DEP), and DEHP, all of which penetrate the placenta[13-15]. In addition, prenatal exposure to phthalates, such as DnBP, BBzP, and DEHP, during the critical window of gestation in male rodents had a reproductively toxic effect on sexual differentiation and caused decreased fertility in offspring[16-23].

In May 2011, the illegal use of DEHP as clouding agent in foods and beverages was reported in Taiwan[24]. It raised the concerns on health effects of phthalates in human, especially in susceptible population. Numerous epidemiological studies

have been conducted to investigate the relationship between phthalates exposure in human and health outcomes, including hormonal regulation of steroid and thyroid hormones, reproductive effects, pregnancy, precocious puberty, obesity and infertility. A systematic overview of published literatures is needed for directing future research and also provides a comprehensive discussion of potential effects in the general population.

MATERIALS AND METHODS

PubMed, Medline and ScienceDirect databases were searched for epidemiological studies focused on phthalates exposure and hormones (thyroid and steroid), reproductive effects, obesity, pregnancy, precocious puberty, and infertility in human. Several potential health effects in human were discussed and listed as follow:

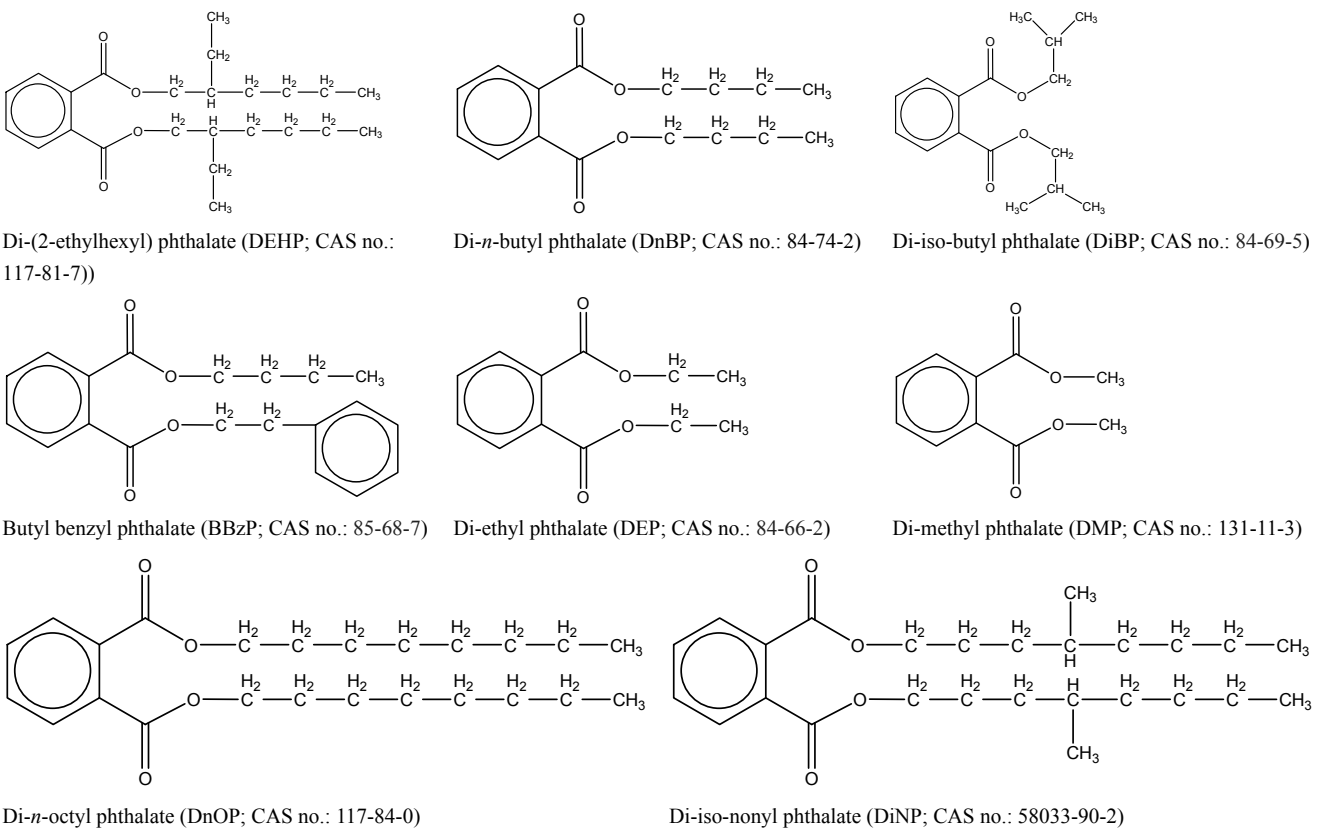
I. Sex Hormone and Reproductive Outcomes
II. Thyroid Hormones
III. Pregnancy Outcomes
IV. Precocious Puberty
V. Obesity
VI. Neurodevelopment
VII. Infertility (Female: Gynecological Diseases; Male: Semen Quality)

The names, chemical structures and abbreviations of phthalates and phthalate metabolites were shown in Figure 1 and Figure 2, respectively.

I. *Sex Hormone and Reproductive Outcomes*

The observation of reproductive health in human studies included sex hormones and examination of reproductive organs. Sex hormones are likely to be affected by the prenatal and postnatal exposure to phthalates (Table 1.1). One study showed that the maternal urinary levels of mono-(2-ethylhexyl) phthalate (MEHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were negatively correlated with the free testosterone (fT) and fT/ estrodial (E_2) levels in cord serum of female newborns[25] and suggested an anti-androgenic effect of phthalates during pregnancy. Another study revealed that mono-*n*-butyl phthalate (MnBP), mono-ethyl phthalate (MEP), and mono-methyl phthalate (MMP) levels in breast milk were significantly positively associated with the luteinizing hormone (LH)/fT and mono-iso-nonyl phthalate (MiNP) with increasing level of LH in infant at three months of age. Besides, MnBP level in breast milk was negatively associated with fT in infant[26].

There is no direct and strong evidence to show the linkage between phthalates exposure and reproductive diseases, such as cryptorchidism, hypospadias or undescended testis. No significant association was found between prenatal phthalates exposure and hypospadias and undescended testis from 284



Di-(2-ethylhexyl) phthalate (DEHP; CAS no.: 117-81-7))

Di-*n*-butyl phthalate (DnBP; CAS no.: 84-74-2)

Di-iso-butyl phthalate (DiBP; CAS no.: 84-69-5)

Butyl benzyl phthalate (BBzP; CAS no.: 85-68-7)

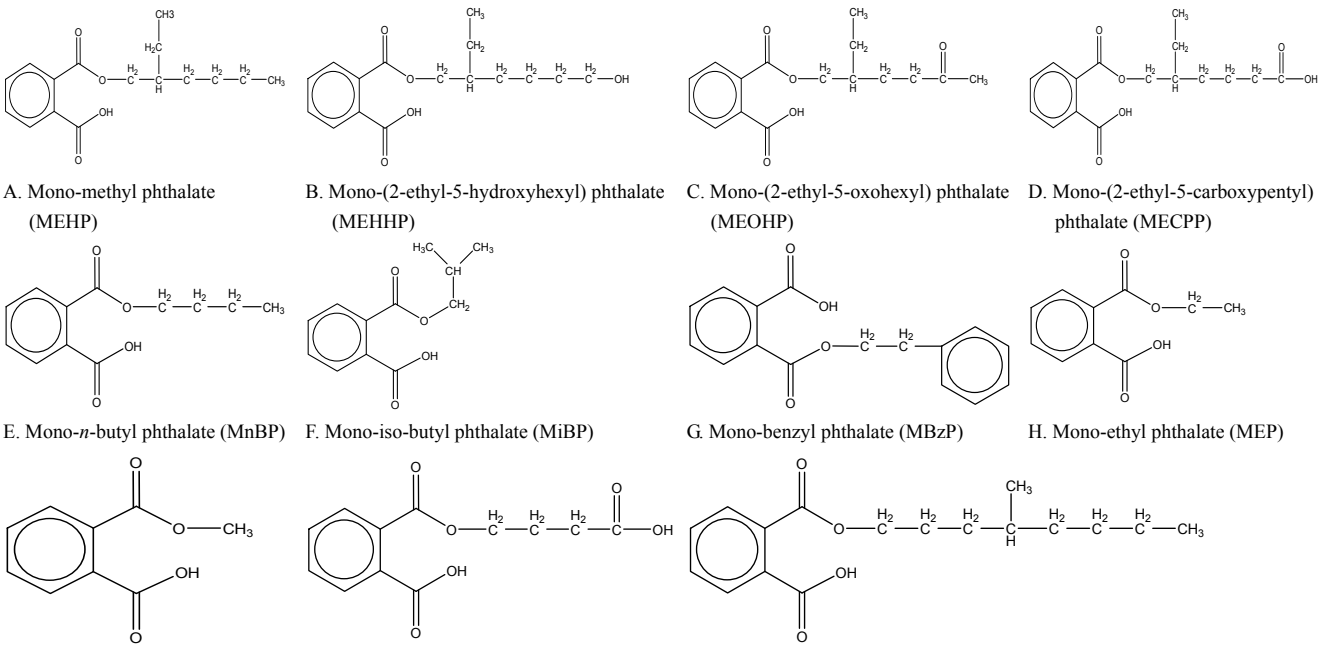
Di-ethyl phthalate (DEP; CAS no.: 84-66-2)

Di-methyl phthalate (DMP; CAS no.: 131-11-3)

Di-*n*-octyl phthalate (DnOP; CAS no.: 117-84-0)

Di-iso-nonyl phthalate (DiNP; CAS no.: 58033-90-2)

Figure 1. Names, numbering and chemical structures of eight phthalates frequently discussed in the reviewed epidemiological studies.



A. Mono-methyl phthalate (MEHP)

B. Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)

C. Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)

D. Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)

E. Mono-*n*-butyl phthalate (MnBP)

F. Mono-iso-butyl phthalate (MiBP)

G. Mono-benzyl phthalate (MBzP)

H. Mono-ethyl phthalate (MEP)

I. Mono-methyl phthalate (MMP)

J. Mono-(3-carboxypropyl) phthalate (MCPP)

K. Mono-iso-nonyl phthalate (MiNP)

Figure 2. Names, abbreviations and chemical structures of phthalate metabolites frequently discussed in the reviewed epidemiological studies. Metabolites of DEHP (A, B, C, D); DnBP (E); DiBP (F); BBzP (G); DEP (H); DMP (I); DnOP (J); DiNP (K).

Table 1.1. Epidemiological studies on phthalates exposure and reproductive hormones/organ

Country / Subjects	Exposure/ Biomarkers	Results	References
Taiwan/ 155 paired pregnant women and newborns	Urine/ MEP, MEHP, MEHHP	In female newborns, the maternal urinary MEP, MEHP, MEHHP: ∝ fT↓ (r=-0.24~ -0.32) MEP, MEHP, MEHHP: ∝ fT/E_2↓ (r=-0.27~ -0.30)	Lin 2011[25]
Denmark/ 130 infants (62 cryptorchid; 68 healthy boys)	Breast milk (1-3 months) MBP, MEP, MMP, MiNP	MEP, MBP: ∝ SHBG ↑ MEP, MMP, MBP: ∝ LH/ fT ↑ MBP: ∝ fT↓(p=0.033) MiNP: ∝ LH ↑	Main 2006[26]
France/ 21 hypospadias and 50 undescended testis boys at birth. 213 matched controls.	Urine/ MEP, MBP, MiBP, MEHP, MEOHP, MEHHP, MECPP, MCNP, MCOP, MBzP, MCPP.	No evidence of association (p_{trend}>0.10) with urinary concentrations of phthalates metabolites was observed for undescended testis risk. Decreased risk of hypospadias, not statistically significant, was observed with urinary concentrations of both low- and high-molecular-weight phthalate metabolites (p_{trend}, 0.13 and 0.10, respectively).	Chevrier 2012[27]

Abbreviations: E_2— estradiol; fT— free testosterone; MBP— mono-butyl phthalate; MEP— mono-ethyl phthalate; MEHP— mono-2-ethyl-hexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; MiNP—mono-*i*-nonyl phthalate; LH—luteinizing hormone; SHBG—sex hormone binding globin.

boys in France[27]. Similarly, no significant association was found between phthalate metabolites levels in breast milk and cryptorchidism in boys[26].

Anogenital distance (AGD) is an indicator of *in utero* androgenic exposure[28] and has been added to the US EPA testing guidelines for reproductive toxicity studies[29-32]. Therefore, AGD has been used as an index for evaluation of androgenic effects in epidemiological studies. In the United States, Swan *et al.* first reported a decrease in AGD among male infants (85 boys aged 2-36 months) with prenatal phthalate exposure[33] (Table 1.2). Four phthalate metabolites, MnBP, mon-iso-butyl phthalate (MiBP), MEP and mono-benzyl phthalate (MBzP) were negatively associated with anogenital index (AGI), AGD divided by birth weight. In a further and multi-center cohort study, collected urine samples from 136 pregnant US women were tested for 9 phthalate metabolites (MnBP, MBzP, mono-(3-carboxypropyl) phthalate (MCPP), MEP, MiBP, MMP, MEHHP, MEHP, mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)) and AGDs were measured in their male infants at 2-36 months[34]. A significant association was reported between AGD and three metabolites of DEHP: MEHHP, MEHP, MEOHP, as well as their sum.

Table 1.2. Epidemiological studies on phthalates exposure and genital development in neonates

Country/ Subjects	Exposure/ Biomarkers	Results	References
Japan/ 111 maternal-infant pairs	Urine/ MEHP	MEHP: ∝ AGI ↓	Suzuki 2011[37]
Taiwan/ 65 maternal-infant pairs	Amniotic fluid & urine/ MBP	MBP: ∝ amniotic fluid ↑ ∝ material urine Amniotic fluid MBP: ∝ AGI↓; AGD ↓	Huang 2009[36]
Mexico/ 73 maternal-infant pairs	Urine/ MEP; MBzP	MEP: ∝ AGD ↓ MBzP∝ penis length and width ↓	Bustamante-Montes 2008[35]
USA/ 134 maternal-infant (boy) pairs	Urine/ MEHP, MEHHP, MEOHP	MEHP, MEHHP, MEOHP: ∝ AGD ↓ , testicular descent	Swan 2008[34]
USA/ 85 maternal-infant (boy) pairs	Urine/ MEP, MnBP, MBzP, MiBP	MBzP, MEP, MnBP, MiBP: ∝ AGI ↓ (OR=3.8, 4.7, 10.2 and 9.1, respectively, all p-values<0.05).	Swan 2005[33]

Abbreviations: AGI— anogential index; AGD— anogenital distance; MBP— mono-butyl phthalate; MBzP— mono-benzyl phthalate; MEP— mono-ethyl phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; MiBP— mono-i-butyl phthalate; MnBP— mono-*n*-butyl phthalate.

Table 2. Epidemiological studies on phthalates exposure and thyroid function

Country/ Subjects	Exposure/ Biomarkers	Results	References
USA/ 1,346 adults (≧20 years) 329 adolescents (12-19 years)	Urine/ MCPP, MECPP, MEHP, MEHHP, MEOHP,	MEHP, MEHHP, MEOHP, MECPP, MCPP: ∝ total T_4 ↓ (P_{trend}<0.001) MEHP, MEHHP, MEOHP, MECPP: ∝ total T_3 ↓ MECPP: ∝ FT_4 ↓ MEHP, MEHHP, MEOHP, MECPP: ∝ TBG ↓ MEHHP, MEOHP, MECPP: ∝ TSH ↑	Meeker 2011[40]
Denmark/ 845 children (4-9 years)	Urine/ MEP, MBP, MBzP, MCiOP, ΣDEHP	∑DEHP, MCiOP: ∝ IGF-1 ↓ (in boys) MEP, MBP, MBzP, MCiOP: ∝ total T_3 ↓ MEP, MBP, MBzP, MCiOP, ∑DEHP: ∝ FT_3 ↓	Boas 2010[41]
USA/ 408 Men (18-55 years)	Urine/ MEHP	MEHP: ∝ FT_4 ↓ ; ∝ T_3 ↓	Meeker 2007[39]
Taiwan/ 76 pregnant women	Urine/ MBP	MBP: ∝ T_4 ↓ ; ∝ FT_4 ↓ (FT_4: β=-0.110, p<0.001; T_4: β=-0.112, p=0.003).	Huang 2007[38]

Abbreviations: DEHP— di-(2-ethylhexyl) phthalate; FT_3— free triiodothyronine; FT_4— free thyroxine; IGF-1—insulin-like growth factor-1; MBP— mono-butyl phthalate; MBzP — monobenzyl phthalate; MCiOP— monocarboxyisooctyl phthalate; MEP— mono-ethyl phthalate; MECPP— mono-2-ethyl-5-carboxypentyl phthalate; MEHP— mono-(2-ethylhexyl) phthalate; MEHHP — mono-(2-ethyl-5-hydroxylhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxo-hexyl) phthalate; T_3— triiodothyronine; T_4— thyroxine; TBG— thyroxine-binding globulin; TSH— thyroid stimulating hormone.

DEHP metabolite was significantly and inversely related to testicular descent.

In Mexico, Bustamante-Montes *et al.* collected urine samples from 73 pregnant Mexican women for four phthalate metabolites (MEHP, MBzP, MEP, MBP) levels[35]. They reported statistically significant and negative association between MEP and AGD, and also between MBzP and penis length and width. In Taiwan, Huang *et al.* collected amniotic fluid samples from 65 pregnant Taiwanese women for five phthalate metabolites levels[36]. They found a significantly negative correlation between amniotic fluid MBP and both AGD (R=-0.31, p<0.05), and AGI (R=-0.32, p<0.05) in girls, and suggested *in utero* exposure to phthalates in general has feminine effects on the fetus. In Japan, Suzuki *et al.* collected urine samples from 111 pregnant Japanese women for seven phthalate metabolite levels[37]. They reported that log-transformed MEHP (specific gravity-corrected) had significantly negative association with AGI and suggested that prenatal exposure to DEHP affects reproductive development in males. Current reviewed studies showed consistent anti-androgenic effects (reproductive hormones and AGD) of DEHP and DBP metabolites in human fetus and newborns.

II. *Thyroid Hormones*

Thyroid hormone is essential for human development of the brain, neurons, growth and development of other organs in children. Hypothyroidism in childhood is accompanied by severe growth retardation, like cretinism. Experimental studies showed potential anti-thyroid effects in certain phthalates. Currently, there are only four studies focusing on this issue in pregnant women[38], adults[39,40] and young children[41] (Table 2). In Taiwan, Huang *et al.* found that urinary MnBP level in pregnant women was significantly negative associated with free thyroxin (FT_4) and thyroxin (T_4) (FT_4: β=-0.110; T_4: β=-0.112, p<0.05), and suggested that exposure to DnBP might affect thyroid activity in pregnant women[38]. In the United States, Meeker *et al.* also reported

a negative association between T_4 and urinary MEHP level in male adults[39]. He further showed significant inverse and dose-dependent relationships between T_4 and urinary DEHP metabolites in adults from the National Health and Nutrition Examination Survey (NHANES) 2007-2008, whereas significant positive relationship between DEHP metabolites and total triiodothyronine (T_3) was found among adolescents[40]. In Denmark, another study found that the levels of urinary phthalate metabolites was associated with thyroid function, insulin-like growth factor I (IGF-I), and growth parameters in children[41]. In girls, phthalate metabolites were negatively associated with serum levels of free and total T_3, while metabolites of DEHP and di-iso-nonyl phthalate (DiNP) were negatively associated with IGF-I in boys. There were some lines of evidence showed possible anti-thyroid activities of DnBP and DEHP in susceptible and general population. Although growing animals and cell models provided supporting evidence on anti-thyroid activities of phthalates, more and large-scale follow-up perspective studies are needed to elucidate the impacts on human health.

III. *Pregnancy Outcomes*

Animal studies indicated that some phthalate metabolites may harm female reproductive function during pregnancy and affect fetal health. Table 3 showed summarized results of epidemiological studies on phthalates exposure and pregnancy outcomes. Latini *et al.* firstly reported newborns (n=84) with cord blood MEHP-positive showed a significantly lower gestational age compared to those with undetectable MEHP[8]. They further showed a significant and positive correlation between detectable MEHP in cord blood and decreased gestational age at delivery (OR=1.50; 95% CI: 1.01-2.21). No significant association with birth weight was found for either DEHP or MEHP in these 84 infants. In the United States, Wolff *et al.* showed that low-molecular-weight (LMW) phthalate metabolites in pregnant women at the third trimester were positively associated with gestational age: 0.97 (95% CI: 0.07-1.9) and head circumference: 0.13 (95% CI: 0.01-0.24)[42]. An increased risk of pregnancy loss (n=48, Hazard Ratio (HR): 2.11; 95% CI: 1.07-4.16) was found among women with MEHP in the upper tertile compared with those in the lowest tertile[43]. The risk of subclinical embryonal loss (n=32) was 16.54 (95% CI: 2.22-123.3) while the risk of clinical spontaneous abortion (n=16) decreased (HR: 0.17; 95% CI: 0.04-0.76). Limited studies showed that phthalate exposure during pregnancy may affect pregnancy loss or gestational age of pregnant women and raise the health concern of fetus or embryo development. Most of studies were conducted with relatively small or limited sample size.

IV. *Precocious Puberty*

The onset of puberty girl included some physical signs, such as enlargement of breast, ovaries, uterus and growth of pubic hair. Precocious puberty, or early onset of puberty, is associated with the development of breast cancer[44] and physical development. Therefore, precocious puberty (before 9 years old) in girls is of great concern. Table 4 showed summarized results of epidemiological studies on phthalates exposure and precocious puberty. In Puerto Rico, Colón *et al.* first reported that levels of phthalates, particularly DEHP, in serum of Puerto Rico girls with premature thelarche (n=41) were significantly higher compared with the control girls (n=35)[45]. In China, DEHP levels in girls with precocious puberty (n=110) were found to be higher compared with the

Table 3. Epidemiological studies on phthalates exposure and pregnancy outcome.

Country/ Subjects	Exposure/ Biomarkers	Results	References
Italy/ 84 newborns	Serum (Cord blood)/ MEHP	MEHP: ∝ gestational age ↓ (OR=1.50; 95% CI: 1.013-2.21; p=0.043)	Latini 2003[8]
USA/ 404 pregnant women	Urine/ MBP, MEP, MiBP, MMP, MEHP, MBzP	Low-molecular-weight phthalate metabolites (low-MWP): ∝ gestational age ↑ (β=0.14, p<0.05); ∝ head circumference ↑ (β=0.13, p<0.05). MEHP: ∝ gestational age ↑ (β=0.15, p<0.05) MEP: ∝ head circumference ↑ (β=0.12, p<0.05) MBzP: ∝ Birth length ↑ (β=0.20, p<0.05)	Wolff 2008[42]
Japan/ 149 pregnant women	Urine/ 9 phthalate metabolites	The relationships between prenatal exposure to phthalate esters and birth outcomes were not significant.	Suzuki 2010[93]
Mexico/ 60 pregnant women	Urine/ MBP, MBzP, MCPP, MEHP, MEHHP, MEOHP, MECPP	After correction by specific gravity or creatinine, geometric mean urinary concentrations were higher for MBP, MBzP, MCPP, MEHP, MEHHP, MEOHP and MECPP among women who subsequently delivered preterm.	Meeker 2009[94]
Denmark, Sweden/ 128 pregnant women	Urine/ MEHP	MEHP: ∝ pregnancy loss ↑ (n=48, OR=2.9; 95% CI: 1.1-7.6).	Toft 2012[43]

Abbreviations: CI— confident interval; MBP— mono-butyl phthalate; MBzP— mono-benzyl phthalate; MCPP— mono-(3-carboxypropyl) phthalate; MEP— mono-ethyl phthalate; MECPP— mono(2-ethyl-5-carboxypentyl) phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; MiBP— mono-*i*-butyl phthalate; OR— odds ratio.

Table 4. Epidemiological studies on phthalates exposure and precocious puberty

Country/ Subjects	Exposure/ Biomarkers	Results	References
Puerto Rico/ 41 thelarche patients & 35 control	Serum/ DMP, DEP, DBP, DEHP,MEHP	DMP, DEP, DBP, DEHP and MEHP: ∝ premature thelarche ↑	Colón 2000[45]
China/ 110 precocious girls & 100 control children	Serum/ DBP, DEHP	In precocious girls: DBP: ∝ uteruses ↑(r=0.456, p<0.05); ovaries ↑(r=0.378, p<0.01). DEHP: ∝ uteruses ↑(r=0.382, p<0.05); ovaries ↑(r=0.689, p<0.01).	Qiao 2007[46]
Taiwan/ 30 girls with premature thelarche, 26 with CPP, and 33 normal controls	Urine/ MBP, MEHP, MMP	MMP: ∝ premature thelarche↑ MBP: ∝ intake of seafood, drink and the use of plastic cups. MEHP: ∝ intake of seafood and meat and exposure plastic handi-wrap.	Chou 2009[47]
USA/ 28 central precocious puberty and 28 age & race-matched prepubertal girls.	Urine/ MnBP, MBzP, MCPP, MECPP,MEHP, MEHPP, MEOHP, MiBP, MEP	No significant differences between the children with central precocious puberty and the controls in either absolute or creatinine normalized concentrations of any of the 9 phthalate metabolites were found	Lomenick 2010[48]
Turkey/ 40 diagnosed pubertal gynecomastia, 21 age-matched control children	Plasma/ DEHP, MEHP	DEHP: ∝ pubertal gynecomastia ↑ (OR=2.77; 95% CI: 1.48-5.21). MEHP: ∝ pubertal gynecomastia ↑ (OR=24.8; 95% CI: 3.5-172.6).	Durmaz 2010[49]
USA/ 1,151 girls (6-8 years)	Urine/ MEP, MnBP, MiBP, MBzP, MCPP, MECPP, MEHHP, MEOHP, MEHP	High-molecular-weight phthalate (high MWP) metabolites were weakly associated with pubic hair development ($PR_{adjusted=}$0.94; 95% CI: 0.88-1.00, fifth vs. first quintile). Small inverse associations were seen for high MWP with pubic hair stage; a positive trend was observed for low MWP biomarkers with breast and pubic hair development. In the first enterolactone quintile, for the association of high BMI with any development, the PR was 1.34 (95% CI, 1.23-1.45 vs. low BMI).	Wolff 2010[50]

Abbreviations: BMI—body mass index; BBP— butyl benzyl phthalate; CI— confident interval; CPP—central precocious puberty; DBP—di-butyl phthalate; DEP— di-ethyl phthalate; DEHP— di-(2-ethylhexyl) phthalate; MBP— mono-butyl phthalate; MBzP— mono-benzyl phthalate; MCPP— mono-(3-carboxypropyl) phthalate; MEP— mono-ethyl phthalate; MECPP— mono-(2-ethyl-5-carboxypentyl) phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; MiBP— mono-i-butyl phthalate; MnBP— mono-n-butyl phthalate; MMP: mono-methyl phthalate; OR— odds ratio; PR— prevalence ratios.

control group (n=100). Girls with higher DEHP levels have larger ovarian and uterus size[46]. In Taiwan, urinary MMP level was significantly higher in premature thelarche group (n=30) than in the control group (n=33)[47]. In the United States, no significant difference in levels of nine phthalate metabolites (MBP, MBzP, MCPP, MECPP, MEHP, MEHPP, MEOHP, MiBP, MEP) was found in central precocious puberty (CPP) (n=28) and prepubertal females (n=28)[48]. In Turkey, Durmaz *et al.* showed that plasma DEHP and MEHP levels were significantly higher in the pubertal gynecomastia group (n=40) compared with the control group (n=21). However, no association was found between plasma DEHP and MEHP levels and any of the examined hormone levels (LH, follicle-stimulating hormone (FSH), E_2, prolactin, thyrotropin, FT_3, FT_4) in Turkey's children[49]. In the United States, a multiethnic longitudinal study of 1,151 girls (6-8 years of age) from five cities was enrolled (2004-2007) to investigate the associations of phthalate exposures with pubertal stages[50]. They found that high-molecular-weight (HMW) phthalate metabolites were weakly associated with pubic hair development and a positive trend was observed for LMW phthalate metabolites with breast and pubic hair development.

Results of case-control and cross-sectional studies indicated that the levels of DEHP or MEHP were significantly higher in the premature thelarch and precocious puberty groups compared with the controls. However, no definite conclusion can be drawn at present, due to lack of consideration of other important factors, such as diet and nutrition.

V. *Obesity*

Obesity is closely linked to metabolic syndrome and numerous diseases, including type II diabetes, cardiovascular diseases, certain cancers and mortality[51]. Experimental studies showed the anti-androgenic effects of phthalate in rodent. Testosterone affects the body fat distribution and decreases the insulin sensitivity in men. Experimental studies in males have shown that testosterone administration reduces lipid uptake by intra-abdominal fat[52] and also reduces visceral fat and improves insulin sensitivity[53-55]. Besides, another possible mechanism is the disruption of thyroid function, which is responsible for the maintenance of basal metabolism. Phthalates are suspected thyroid disruptors and

Table 5. epidemiological studies on phthalates exposure and obesity.

Country/ Subjects	Exposure/ Biomarkers	Results	References
USA/ 1,451 adult male (>18 years) from NHANES 1999-2002	Urine/ MEP, MBP, MBzP, MEHP, MEHHP, MEOHP	MBP, MBzP, MEP: ∝ HOMA ↑ MBzP, MEP, MEHHP, MEOHP: ∝ waist circumference ↑	Stahults 2007[57]
USA/ 4,369 adults (>20 years) and some adolescent girls from NHANES 1999-2002	Urine/ MBzP, MEHP	Phthalate metabolites: ∝ BMI↑; waist circumference↑(in males) MBzP: ∝ BMI ↑ (in males aged 20-59) MEHP: ∝ BMI ↑ , waist circumference ↑ (in female aged 12-59 years old)	Hatch 2010 [58]

Abbreviations: BMI—body mass index; HOMA— homeostatic model assessment; MBP— mono-butyl phthalate; MBzP— mono-benzyl phthalate; MEP— mono-ethyl phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate.

may reduce circulating thyroid levels[56]. There is a growing concern about the effects of phthalates on obesity and cardiovascular disease.

Table 5 showed summarized results of phthalates exposure and obesity in epidemiological studies. Stahults *et al.* firstly reported four metabolites (MBzP, MEHHP, MEOHP, and MEP) were associated with increased waist circumference (WC) and three with increased HOMA (MBP, MBzP, and MEP) from US male adults (NHANES 1999-2002, n=1451) [57]. They suggested that exposure to these phthalates may contribute to the population burden of obesity, insulin resistance, and related clinical disorders. Another study presented their findings between levels of six phthalate metabolites and body mass index (BMI) and WC using data from NHANES[58]. They found positive associations between BMI and WC among adult males for most phthalate metabolites, like MBzP. In females, BMI and WC increased with quartiles of MEP in 12-19 year olds, and a similar but less strong pattern was seen in 20-59 year olds. By contrast, higher levels of MEHP were associated with lower BMI in adolescent girls and females aged 20-59. Only little evidence revealed possible linkage between phthalate exposure and obesity in adults and adolescence. No definite conclusion can be drawn at present due to being devoid of other important factors, such as diet, smoking, activity and alcohol consumption.

VI. *Neurodevelopment*

Laboratory experiments with rodents have revealed that higher levels of DEHP may have adverse effects on neurobehavioral parameters. While the widely application and exposure of phthalates in human, the adverse effects of phthalates on children's neurodevelopment raise the public health concern, such as intelligence quotient (IQ), behavior and attention-deficit/hyperactivity disorder (ADHD). Some epidemiological studies have examined phthalates exposure in relation to neurodevelopment among neonates after birth in United States and Mexico[59-61], in Korea in children from elementary schools[62-64] and in United States both among children 4-9 years[65] and 3.6-6.4 years of age[66] (Table 6).

In the United States, a study was performed among neonates enrolled in a multiethnic birth cohort at the Mount Sinai School of Medicine; urinary concentrations of phthalate metabolites in pregnant women and neonatal behaviors measured within 5 days of birth using the Brazelton Neonatal Behavioral Assessment Scale were assessed. They showed strong and inverse associations between increasing concentrations of HMW phthalate metabolites (MBzP, MECPP, MEHHP, MEOHP, MEHP, MCPP) and orientation scores among girls (n=295)[59]. Another multicenter pregnancy cohort study prospectively evaluated the influence of prenatal phthalate exposure on children's behavior in the pre-school age. They found a decreased composite score (less masculine) in boys (n=74) was associated with increasing concentration of MnBP, MiBP and their sum[66]. In South Korea, DEHP metabolites were significantly associated with ADHD in 261 children aged 8-11 years. DBP metabolites were significantly associated with number of omission and commission errors in continuous performance test[62]. Another cross-sectional study conducted by Cho *et al.* reported that full-scale IQ and verbal IQ of school-age children (around 9 years, n=667) were negatively associated with DEHP metabolites, especially in boys[63]. A birth cohort study conducted by Kim enrolled 460 mother-infant pairs from three South Korean cities. They found that Mental Development Index (MDI) was significantly and inversely associated with the natural logarithm levels of MEHHP (β=-0.97; 95% CI: -1.85, -0.08) and MEOHP (β=-0.95; 95% CI: -1.87, -0.03); and Psychomotor Development Index (PDI) was inversely associated with MEHHP (β=-1.20; 95% CI, -2.33, -0.08), especially in boys[64]. No significant linear association was observed for girls.

In the United States, Whyatt *et al.* found that Child PDI scores decreased with increasing $\log_e$ MnBP ($\beta_{adj.}$=-2.81, 95% CI: -4.63, -1.0) and $\log_e$ MiBP ($\beta_{adj.}$=-2.28, 95% CI: -3.90, -0.67); odds of motor delay increased significantly ($OR_{adj.}$)=1.64 (95% CI: 1.10, 2.44) and 1.82 (95% CI: 1.24, 2.66 per $\log_e$ MnBP and $\log_e$ MiBP). MDI scores decreased with increasing $\log_e$ MnBP (β=-2.67, 95% CI: -4.70, -0.65) only in girls; the child sex difference in odds of mental delay was significant. The OR's for clinically withdrawn behavior were 2.23 (95% CI: 1.27, 3.92) and 1.57 (95% CI: 1.07, 2.31) per

Table 6. Epidemiological studies on phthalates exposure and children's neurodevelopment

Country/ Subjects	Exposure/ Biomarkers	Results	References
USA/ 295 children and pregnant women	Urine/ MMP, MEP, MnBP, MiBP, MBzP, MECPP, MEHHP, MEOHP, MEHP, MCPP	High-MWP metabolites: ∝ Orientation score ↓ ; Alertness score ↓ ; ∝ Neurodevelopment ↓ (p=0.02). High-MWP metabolites: ∝ Neurodevelopment ↓	Engel 2009[59]
USA/ 74 boys and 71 girls (3.6-6.4 years of age)	Urine/ MEHHP, MEOHP, MnBP, MiBP	MEHHP, MEOHP, MnBP, MiBP: ∝ masculine score ↓ (In boys).	Swan 2010[66]
South Korea/ 261 children, age: 8-11 years	Urine/ MEHP, MEHHP, MEOHP	MEHP, MEHHP, MEOHP: ∝ ADHD scores ↓	Kim 2009[62]
South Korea/ 667 children at nine elementary schools in five South Korean cities	Urine/ MEHP, MEOHP, MnBP	MEHP, MEOHP, MnBP: ∝ WISC vocabulary score ↓ (In boys)	Cho 2010[63]
South Korea/ 460 mother–infant pairs (2006 and 2009)	Urine/ MEHHP, MEOHP, MnBP	MEHHP, MEOHP: ∝ MDI scores↓(β=-0.97, -0.95) MEHHP: ∝ PDI scores↓(β=-1.20) MEHHP, MEOHP, MnBP: ∝MDI↓(in males, β=-0.93~ -1.57) MEHHP, MEOHP, MnBP: ∝ PDI↓(in males, β=-1.25~ -2.36)	Kim 2011[64]
USA/ 319 pregnant women	Urine/ MBzP, MiBP, MnBP	MiBP, MnBP: ∝ PDI scores ↓ ($\beta_{adj.}$=-2.81; -2.28) MnBP: ∝ MDI scores ↓ (in girls, β=-2.67) MnBP, MBzP: ∝ clinically withdrawn behavior (β=2.23; 1.57) MBzP: ∝ clinically internalizing behaviors(β=1.43)	Whyatt 2012[60]
Mexico/ 45 children (20 boys and 25 girls); age: 36 months	Urine/ MEHP, MBzP	MEHP, MBzP: ∝ MDI scores ↓ (β=-5.53; -5.11, in girls)	Téllez-Rojo, 2011[61]

Abbreviations: CI— confident interval; MBP— mono-butyl phthalate; MBzP— mono-benzyl phthalate; MCPP— mono-(3-carboxypropyl) phthalate; MDI—mental developmental index; MEP—mono-ethyl phthalate; MECPP—mono- (2-ethyl-5-carboxypentyl) phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; MiBP— mono-*i*-butyl phthalate; MMP— mono-methyl phthalate; MnBP: mono-*n*-butyl phthalate; OR— odds ratio; PDI— psychomotor development index.

$\log_e$ unit increase in MnBP and MBzP, respectively; for clinically internalizing behaviors, the OR was 1.43 (95% CI: 1.01, 1.90) per $\log_e$ unit increase in MBzP. They found significant child sex differences in associations between MnBP and MBzP and behaviors in internalizing domains (p<0.05)[60].

In Mexico, Téllez-Rojo *et al.* found that an increase in $\log_e$ concentrations of MEHP and MBzP was associated with lower MDI scores in girls (n=25) at 36 months of life after adjustment for potential confounders. However, they found no statistically significant association between MDI and prenatal phthalate metabolites levels among boys (n=20). They suggested a gender differential effect of prenatal phthalates exposure in relation to children's neurodevelopment[61]. The results from most of presented studies indicated possible linkage between phthalate exposure and cognitive function in toddler and young children. Prenatal and postnatal phthalate exposure may inversely associate with child's neurodevelopment. It is necessary to evaluate the neurological effects of other exposure, such as lead, mercury and manganese in future studies

VII. *Infertility (Female: Gynecological Diseases; Male: Semen Quality)*

(I) *Female Gynecological Diseases*

Endometriosis, adenomyosis and leiomyomas are common gynecologic disorders presented by prolonged or heavy menstrual bleeding, pelvic pain and infertility. The prevalence of endometriosis has been reported to be 2-22% in women of childbearing age, whereas adenomyosis and leiomyomas have a prevalence of 20-35% in the infertility clinic and 20-25% in premenopausal women[67-69]. Previous studies have revealed that some extensively used phthalates, like DEHP, are possibly associated with endometriosis[70-72].

Table 7.1 showed summarized results of epidemiological studies on phthalates exposure and female infertility. In Italy, Cobellis *et al.* showed that endometriotic women (n=55) had significantly higher plasma DEHP levels than controls (n=24) and 92.6% of them had detectable DEHP or MEHP in the peritoneal fluid[70]. However, another study with a smaller sample size reported that women with uterine fibromatosis (n=15) had significantly lower MEHP levels than controls (n=20)[73]. In India, some studies reported that women with endometriosis had significantly higher DnBP, BBzP, DEHP, dimethyl phthalate (DMP), DEP and di-*n*-octal phthalate (DnOP) levels than controls and these exposure levels

Table 7.1. Epidemiological studies on phthalates exposure and female gynecological diseases

Country/ Subjects	Exposure/ Biomarkers	Results	References
Italy/ 55 Endometriosis, 24 age-matched control women.	Plasma/ DEHP	DEHP concentrations: ∝ Endometriotic women ↑	Cobellis 2003[70]
India/ 49 infertile women with endometriosis, 38 control group I; 21 control group II.	Plasma/ DnBP, BBP, DEHP, DnOP	DnBP, BBP, DnOP, DEHP: Endometriosis women ↑ DnBP, BBP, DnOP, DEHP: ∝ Endometriosis ↑ (r=0.44~0.78)	Reddy 2006a[71]
Italy/ 15 uterine fibromatosis, 20 control	Serum/ DEHP, MEHP	MEHP: Fibromatosis group ↓ (p<0.01) DEHP: Fibromatosis group ↓ (p<0.01)	Luisi 2006[73]
India/ 97 endometriosis 102 control	Blood / DnBP, BBP, DEHP, DnOP	DnBP, BBP, DnOP, DEHP: Endometriosis group > control group GSTM1 deletion: endometriosis ↑ (OR=2.12, 95% CI:1.045-4.314, p=0.03)	Roya 2009[75]
India/ 99 endometriosis; 135 control	Serum/ DMP, DEP, DnBP, BBP, DEHP	DMP, DEP, DnBP, BBP, DEHP: Endometriosis group ↑ (p<0.001) DMP, DnBP, BBP, DEHP: ∝ Endometriosis ↑ (r=0.33~0.89)	Roya 2008[74]
Japan/ 137 endometriosis: 80 control (Stages0-1); 57 case (Stages2-4)	Urine/ MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP	No significant association between endometriosis and any urinary reatinine-adjusted phthalate metabolites was found. $OR_{adjusted}$ for higher dichotomized MEHP by endometriosis was 1.57 (95% CI: 0.74-3.30). No monotonic trend was found in urinary concentration of phthalate metabolites by endometriosis stage (p=0.23-0.90).	Itoh 2009[76]
Taiwan/ 28 endometriosis, 16 adenomyosis, 36 leiomyomas, 29 controls	Urine/ MnBP, MEHP, ∑MEHP (MEHP+ MEHOP+ MEHHP)	∑MEHP: Leiomyomas women ↑ (p<0.05) MnBP: Endometriosis women ↑ (p<0.05) ∑MEHP: ∝ Adenomyosis, Leiomyoma ↑ (OR=10.4; 5.93)	Huang 2010[77]
USA/ 1,227 women self-reported history of endometriosis and uterine leiomyomata (NHANES).	Urine/ MBP, MEHP	MBP: ∝ endometriosis, leiomyomata ↑ (OR=1.36, 1.56) MEHP: ∝ endometriosis, leiomyomata ↓ (OR=0.44, 0.63)	Weuve 2010[78]

Abbreviations: BBP— butyl benzyl phthalate; CI— confident interval; DEP— di-ethyl phthalate: DEHP— di-(2-ethylhexyl) phthalate; DMP— di-methyl phthalate; DnBP— di-*n*-butyl phthalate; DnOP— di-*n*-octyl phthalate; GSTM1— Glutathione S-transferase M1; MBP— mono-butyl phthalate; MBzP— mono-benzyl phthalate; MEP— mono-ethyl phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; MnBP— mono-*n*-butyl phthalate; ORs— odds ratio.

was strongly correlated with the severity of endometriosis, and gene polymorphism of glutathiones S-transferase M1 (GSTM1) might be a risk factor for endometriosis[71,72,74,75]. In Japan, Itoh *et al.* neither found significant association between endometriosis (n=57) and five phthalate metabolites (MEHP, MEOHP, MEHHP, MnBP, and MEP) nor positive trend between phthalate exposure and severity of endometriosis[76]. In Taiwan, Huang *et al.* showed that patients with leiomyoma (n=36) had significantly higher levels of sum DEHP metabolites (MEHP, MEHHP and MEOHP) than controls (n=29), whereas those with endometriosis (n=28) had an increased level of MnBP. They further found that subjects who carried the GSTM1 null type with a high urinary level of sum DEHP metabolites showed a significantly increased risk for adenomyosis (OR=10.4, 95% CI: 1.26-85.0) and leiomyomas (OR=5.93, 95% CI: 1.10-31.9) after adjustment for age[77]. In the United States, Weuve *et al.* found positive association for MBP and inverse association for MEHP in relation to endometriosis and leiomyoma, respectively, from NHANES 1999-2004[78]. Several case-control and cross-sectional studies have provided a possible linkage between phthalates exposure and female infertility. A long-term and large-scale prospective study of girls exposed *in utero* to phthalates would be needed to answer whether maternal exposure to phthalates had bearings on estrogen regulation and epi-genetic change in the offspring.

(II) *Male Semen Quality*

Experimental studies have reported that phthalates can adversely affect sperm quality in rodents and increasing epidemiological studies have revealed reproductive effects, including sperm activity and spermatogenesis, of phthalates on male infertility (Table 7.2). In the United States, Duty *et*

al. showed an inverse dose-response relationship between two phthalate metabolites (MBP, MBzP) and sperm activity (sperm concentration and motility) from 168 subfertile couples in Boston[79]. His team further enlarged a smaller study of 463 infertile men and extended to the Great Lakes Region including 45 infertile men[80]. They further showed that an interquartile range (IQR) change in MBzP exposure was significantly associated with a 10% decrease in FSH concentration and that MBP exposure was borderline-significantly associated with a 4.8% increase in inhibin B[81]. Mendiola *et al.* recruited 425 male adults to evaluate the effects of 11 phthalate exposure on reproductive hormones. They found that total testosterone, calculated free testosterone and the free androgen index (FAI) were inversely correlated with the urinary levels of four DEHP metabolites. Only FAI was significantly associated with the urinary concentrations of several DEHP metabolites, after adjustment by appropriate covariates. Besides, sex hormone binding globulin (SHBG) was positively related to the urinary concentration of MEHP after adjustment[82].

In India, semen DEHP metabolites' level in infertile men was negatively correlated with sperm concentration, motility and percentage (%) of abnormal sperm[83]. In Sweden, Jonsson *et al.* (2005) showed that young men (n=234) within the highest quartile of MEP had fewer motile sperms, more immotile sperms, and lower LH values[84]. In China, there was a significant positive association between liquefied time of semen and phthalate (DEP, DBP, DEHP) concentrations of semen, whereas there was no significant relation between phthalate concentrations and sperm density and livability[85]. A total of 150 participants (125 men and 25 women) were recruited from couples seeking fertility assessment from the Reproduction Department of the Chongqing Institute of Science and Technology for Population and Family Planning (CISTPF)[86]. They reported average concentrations for MMP, MEP, MBP, MBzP, MEHP and MEOHP were 41.3, 300, 41.0, 0.78, 2.99 and 3.90 μg/g creatinine, respectively, in these subjects. They found a borderline-significant dose–response relationship between MBP and sperm concentration, with ORs of 1.0, 6.8 and 12.0 for increasing exposure tertiles (p=0.05), after adjustment. A positive correlation between MEP and straight-line velocity of sperm motion was observed without statistical significance.

Results of several studies of subfertile men demonstrated associations between phthalate levels and impaired sperm quality (i.e. sperm concentration, morphology, motility), but most in the infertility male adults. In addition, long term prospective studies of *in utero* exposure to phthalates in boys would be needed to answer whether maternal exposure to phthalates had bearings on semen quality in the offspring.

DISCUSSION

Results of the reviewed studies indicated that phthalates exposure may affect reproductive hormones[25-27] and developmental outcomes[33-37], thyroid hormones[38-41], pregnancy outcomes[8,42,43,93,94], precocious puberty[45-50], neurodevelopment[59-64,66], male infertility[79-86,95-98] and female gynecological diseases[70,71,73-78] (Figure 3). At least one of phthalates metabolites were associated with above health outcomes in reviewed studies. Exposure to DEHP metabolites (MEHP, MEHHP, MEOHP) was negatively associated with free testosterone[25,26,82], ano-genital distance[34,37], gestational age[8], thyroid function (T_4, T_3, TBG)[39-41], IGF-I[41], children' neurodevelopment[59], ADHD[62], DNA damage[97] and sperm quality (concentration, motility, morphology) [80,83,96,98]. On the other hand, exposure to

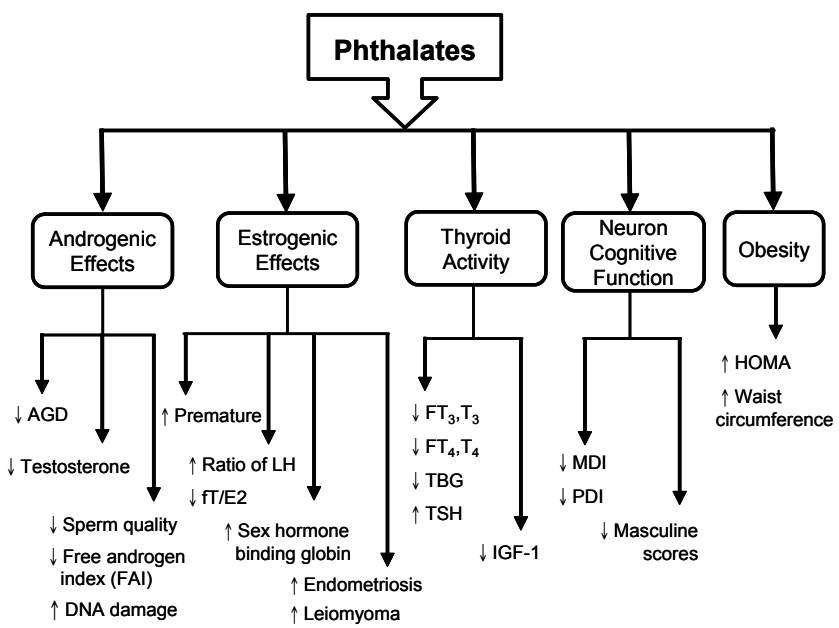


Figure 3. Summarized endocrinal effects of phthalates exposure in human studies.

DEHP metabolites was positively associated with precocious puberty[45-47], size of uterus and ovarian[46], waist circumference[57,58], female gynecological diseases (endometriosis and leiomyoma)[70,71,74,77,78]. Other phthalates metabolites (MEP and MBP) were also positively correlated with SHBG[26] and ratio of LH[26] and impacted on T_4[38], FT_4[38], T_3[41], FT_3[41],

Table 7.2. Epidemiological studies on phthalates exposure and male infertility

Country/Subjects	Exposure/ Biomarkers	Results	References
USA/ 168 men (subfertile) from the hospital	Urine/ MEP	MEP: ∝ comet extent↑ (3.6 μm, 95% CI: 0.74-6.47, p=0.015) MEP: ∝ TDM↑ (1.2 μm, borderline significance) MBP, MBzP, MMP and MEHP were not significantly associated with any comet assay parameters.	Duty 2003[79]
USA/ 168 men (subfertile) from the hospital	Urine/ MBP, MBzP	MBP_ tertile: ∝ Sperm motility ↑ (OR: 1.0, 1.8, 3.0; p=0.02) ; ∝ Sperm concentration ↑ (OR:1.0, 1.4, 3.3; p=0.07) MBzP_ tertile: ∝ sperm concentration ↑ (OR:1.0, 1.4, 5.5; p=0.02)	Duty 2003[95]
USA/ 295 male (subfertile) from the hospital	Urine/ MBP, MBzP	MBzP: ∝ FSH concentration ↓ (10%, p=0.003) MBP: ∝ inhibin B ↑ (4.8%, p=0.07)	Duty 2005[81]
Sweden/ 234 young Swedish men	Urine/ MEP	MEP: ∝ Sperm motility↓; LH values↓	Jönsson 2005[84]
USA/ 463 subfertile male	Urine/ MBP, MBzP	MBP, MBzP: ∝ Sperm concentration ↓ MBP: ∝ Sperm motility ↓	Hauser 2006[96]
China/ 52 men (23- 48 years)	Semen/ DEP, DBP, DEHP	DEP, DBP, DEHP: ∝ Liquefied time of semen (r=0.456,0.475, 0.457, p=0.01) There was no significant difference between phthalate concentrations of semen and sperm density or livability.	Zhang 2006[85]
USA/ 463 men (subfertile) the hospital.	Urine/ MEHP	MEHP: ∝ DNA damage ↑ MEHP: ∝ Comet extent ↑ (17.3%); ∝ TDM ↑ (14.3%); ∝ Tail% ↑ (17.5%)	Hauser 2007[97]
USA/ 45 subfertile men	Urine/ MEP, MCPP, ΣDEHP	MEP: ∝ Sperm concentration ↓ (OR=6.5, p=0.05) MCPP: ∝ Low morphology ↓ (OR=7.6, p=0.05) ∑DEHP: ∝ Sperm concentration ↓ (OR=5.4, p=0.05) MEP: ∝ Low morphology ↓ (OR=3.4, p<0.05)	Wirth 2008[80]
India/ Healthy human males (21–40 years)	Semen/ DEP, DEHP, DBP	DEP, DBP, DEHP: ∝ Sperm concentration ↓ (p<0.05) (r=-0.19, -0.20, -0. 25) DBP, DEHP: ∝ Sperm motility ↓ (p<0.05) (r=-0.18) DEHP: ∝ Abnormal sperm ↑ ; Depolarized mitochondria ↑ ; LPO ↑ (p<0.05) (r=0.18, 0.23, 0.20) DBP, DEHP: ∝ DFI ↓ (r=0.18, 0.20; p<0.05) DBP, DEHP: ∝ ROS ↓ (r=0.19, 0.20; p<0.05)	Pant 2008[83]
USA/ 425 men (with a pregnant women)	Urine/ MEHP, MEHHP, MEOHP, MECPP	MEHP, MEHHP, MEOHP, MECPP: ∝ FAI ↓ (p<0.05) (r=-0.23, -0.14, -0.15, -0.12) MEHP, MEHHP, MEOHP, MECPP: ∝ FT ↓ (p<0.01) (r=-0.15, -0.13, -0.14, -0.13) MEOHP, MECPP: ∝ TT ↓ (r=-0.10, -0.10; p<0.05) MEHP: ∝ FAI/ LH ratio ↓ (r=-0.13; p<0.01) MEHP: ∝ SHBG ↑ (r=0.14, p<0.01)	Mendiola 2011[82]
China/ 125 men and 25 women	Urine/ MEP, MBP	MBP_ tertile: ∝ Sperm concentration ↑ (ORs:1.0, 6.8, 12.0; p=0.05) MEP: ∝ Sperm motion (VSL) ↑ (r=0.232, p=0.05)	Liu 2011[86]
Taiwan/ 45 male workers (PVC pellet plants)	Air/ DEHP	DEHP: ∝ Sperm DNA denaturation (αT) ↑ ; DFI ↑ (β=0.038; 0.140, p<0.05) DEHP: ∝ Sperm motility ↓ (β=−0.227, p<0.05)	Hsu 2011[98]

Abbreviations: CI— confident interval; DBP— di-butyl phthalate; DEP— di-ethyl phthalate ; DEHP— di-(2-ethylhexyl) phthalate; DFI—DNA fragmentation index; FAI— free androgen index; FSH—follicule stimulating hormone; FT— free testosterone; IQR— interquartile range; LH— luteinizing hormone; LPO— lipid peroxidation; MBP— monol-butyl phthalate; MBzP— mono-benzyl phthalate ; MCPP— mono-(3-carboxypropyl) phthalate; MEP— mono-ethyl phthalate; MECPP— mono-2-ethyl-5-carboxypentyl) phthalate; MEHP— mono-2-ethylhexyl phthalate; MEHHP— mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP— mono-(2-ethyl-5-oxohexyl) phthalate; ORs— odds ratio; PVC—polyvinylchloride; ROS— reactive oxygen species; SHBG— sex hormone binding globin; αT —sperm DNA denaturation; TDM— tail distributed moment; TT— testosterone; VSL— straight-line velocity

AGD[33,35,36], semen quality[86], DNA damage[79,84,95,98] and children's neurodevelopment[59,60,64,66].

Results of the reviewed studies were all adjusted for important confounders by adequate statistical method. In studies of phthalates exposure and reproductive outcomes[25-27,33-37] were adjusted for: birth weight and gender of newborns or infants, gestational age, race, maternal education, BMI and smoking during pregnancy. In thyroid studies[38-41]: age, BMI, gestational age, race, gender, smoking status, TBG. In pregnancy studies[8,42,43,93,94]: gender of newborns, birth order, gestational age, maternal BMI, maternal age, maternal smoking status. Results of studies on children's neurodevelopment[59-64,66] were adjusted for: age, gender of child, maternal education, mother's IQ and socioeconomic status. Results of studies on semen quality[79-86,95-98] were adjusted for: age, BMI, smoking status whereas in female gynecological diseases[70,71,73-78] on age, BMI, race, GSTM1, age at menarche, current pregnancy and breastfeeding status.

Exposure assessment of phthalates plays an important role of most of epidemiological studies. As phthalates are metabolized to their metabolites within a few hours or days[87,88], urinary phthalate metabolites are considered good biomarkers for assessing phthalate exposure in humans because of their low contamination rate in the laboratory and reliability for indicating an individual's phthalate exposure[88,89]. Some studies[89-91] showed good reliabilities, specifies and representative of one single spot urine for prediction of individual's phthalate exposure over 3 - 6 months. Some studies[8,45,46,49] assessed human phthalates exposure in cord blood, plasma and serum. However, using serum or plasma sample may not be the best exposure assessment of phthalate because of laboratory contamination[14] and rapid metabolization to their metabolites[88]. For evaluation of critical points of prenatal phthalate exposure, amniotic fluid sample could be better than cord blood[8,36].

Most of the reviewed studies were cross-sectional or case-control design to assess the phthalates exposure and health impact of human. However, the interpretations of these results may boundary due to some limitations, such as uncertainty of causal relationship, selection and recall bias. Therefore, there is a need to conduct large-scale longitudinal studies in the future . Such studies should have sufficient sample size, representative populations and also collect the influence of confounding factors.

CONCLUSIONS

In reviewing of current epidemiological studies, we suggest that phthalates may alter reproductive and sex hormones, thyroid function, development and neurodevelopment. Although more and large-scale epidemiological studies are needed to clarify their association, reducing the phthalate exposure in pregnant women, newborns and children is necessary to prevent unexpected consequences of reproductive and neurodevelopment effects in our offspring.

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