The Turkish Journal of Pediatrics 2022; 64: 187-209
https://doi.org/10.24953/turkjped.2020.2060

Review

Maternal phthalate exposure during pregnancy and male reproductive disorders: a systematic review and meta-analysis

Chengjun Yu[1,2,3], Jiandong Lu[1,2,3], Jie Zhao[1,2,3], Tianxin Zhao[1,2,3,4], Chunlan Long[3,4,5], Tao Lin[1,2,3], Shengde Wu[1,2,3,5,6], Sheng Wen[1,2,3,5,6], Guanghui Wei[1,2,3,5,6]

[1]Department of Urology, Children's Hospital of Chongqing Medical University, Chongqing, China; [2]Chongqing Key Laboratory of Children Urogenital Development and Tissue Engineering, Chongqing, China; [3]National Clinical Research Center for Child Health and Disorders, Chongqing, China; [4]Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing, China; [5]China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing, China; [6]Chongqing Key Laboratory of Pediatrics Chongqing, Chongqing, China.

ABSTRACT

Background. Phthalates are ubiquitous in the environment and they can penetrate the human body via multiple routes. However, the impact of phthalates on human male reproductive disorders remains unclear.

Methods. A critical review of published studies was conducted to clarify the association of phthalates and male reproductive disorders and to highlight future research needs. PubMed, Cochrane Library, and Web of Science Database were systematically searched for relevant articles written in English, independent of region and time period. If more than one paper overlapped in study design or participants included, the most recent manuscript was included in our review. Due to limited homogeneous statistical data, observed trends were summarized to draw approximate conclusions.

Results. Nineteen manuscripts were included in our final analysis. Exposure to di-(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and/or benzyl butyl phthalate (BBP) is associated with a shorter anogenital distance (AGD). Meanwhile, exposure to DEHP and/or di-isodecyl phthalate (DIDP) is associated with higher risks for cryptorchidism and hypospadias.

Conclusions. Generic exposure to phthalates has an adverse effect on human reproductive development, especially exposure to DEHP, DBP, DEP, BBP, and DIDP. A critical time for exposure sensitivity is during early pregnancy. Due to the lack of significant statistical power in this study, the conclusions drawn should be cautiously interpreted and they remain to be validated. Thus, additional well-designed studies, as well as propaganda and education regarding phthalate exposure and safer substitutes for these compounds, are greatly needed.

Key words: anogenital distance, cryptorchidism, hypospadias, male reproductive disorders, phthalates.

Phthalates are a class of manufactured chemicals that are commonly used to enhance the flexibility of plastics. By far, the major use of phthalates is as plasticizers in the production of polyvinyl chloride (PVC) products. PVC is the second most commonly used plastic in the world based on its use in pipes, construction materials, electronic wiring, and thousands of other applications. Phthalates are also used as additives in various consumer products, including food packaging, toys, shoes, cosmetics, and skin care products. Furthermore, phthalates are included in some medications and pesticides.[1]

✉ Shengde Wu and Sheng Wen
shengdewu@hospital.cqmu.edu.cn

Received 31st July 2020, revised 12th June 2021, accepted 6th July 2021.

Phthalate-containing products are quite common in daily life, yet they are not covalently bound to a product's matrix. Therefore, it is possible for phthalates to be released into the environment. As a result, phthalates have routinely been found to contaminate indoor air, dust, food, and water.[2-4] The major route of phthalate exposure is via ingestion of phthalate-contaminated food. However, phthalates can also permeate our body via dermal and inhalation pathways.[5]

Phthalates are endocrine disrupting chemicals (EDCs) which have been associated with male reproductive disorders (MRDs) in animal models over the past 20 years. For example, phthalates have been shown to act as anti-androgens and to downregulate the production of testosterone.[6,7] In rats, this is described as, "Phthalate Syndrome". Following *in* utero exposure to anti-androgen phthalates, a decrease in testosterone levels leads to an increased risk of conditions such as cryptorchidism and hypospadias.[7,8] Rodent studies have also identified that phthalates, especially di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP), can negatively affect androgen signaling when administered during a critical period of development in the genitourinary tract.[9,10] Furthermore, antenatal exposure to phthalates [notably DEHP, DBP, and butyl benzyl phthalate (BBzP)] induces a shorter anogenital distance (AGD) in rodents.[10,11]

To date, only a few studies have explored the relationship between prenatal phthalate exposure and the development of MRDs in humans. As a result, knowledge regarding this possible correlation remains incomplete. A key finding was made by Jensen et al.[12] who detected phthalate metabolites in amniotic fluid, thereby indicating the capacity for these compounds to cross the placental barrier and expose fetuses to their effects. Newborns are also vulnerable to endocrine disruption by chemicals, especially during the period of masculinization programming. The latter process of male sexual differentiation is driven by gonadal hormones. Here, we conducted a systemic review and meta-analysis to summarize the information currently available, to clarify associations between phthalate exposure and higher risks of cryptorchidism and hypospadias, as well as a shorter AGD, and to highlight future directions for studies of phthalates and their health risks.

Methods

Definition of MRDs and phthalate exposure

MRDs examined

Cryptorchidism refers to the testes being located somewhere along the path from the waist retroperitoneum down to the scrotum, yet not in accordance with the descent that normally occurs. Hypospadias is defined as displacement of the urethral meatus from the tip of glans penis to the ventral side of the phallus, scrotum, or perineum.[13] These conditions represent the two most common types of congenital defects that lead to abnormalities in male genitalia.[14,15] AGD is the distance from the center of the anus to an external genital landmark. In males, the external genital landmarks can include: (1) the base of the scrotum where the skin changes from rugated to smooth (AGDas); (2) the posterior or caudal insertion of the penis (AGDapp), and (3) the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone (AGDap).[16-18] Details regarding measurements of AGD are provided in Table I. AGD has been shown to serve as a readout of androgen concentrations during prenatal development in mammals.[19] Moreover, a shorter AGD in males is typically associated with higher risks for cryptorchidism and hypospadias. Since the AGD is easy to measure, it can have significant utility in clinical evaluations and epidemiological research studies. In this study, we identified risk of cryptorchidism, risk of hypospadias, and AGD as outcome parameters to assess the effect of prenatal phthalate exposure on MRDs.

Exposure to phthalates

Prenatal exposure to phthalates was defined as epidemiological exposure to generic phthalates

Table I. Anogenital distance (AGD) and exposure to phthalates (defined as urinary phthalate metabolites concentrations).

Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Definition and assessment of anogenital distance (AGD) (time of assessment)	Type of biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
Adibi 2015	Partly eligible subjects from The Infant Development and Environment Study (TIDES), prospective birth cohort	Maternal urine PMC detected by isotope-dilution tandem detection by isotope-dilution tandem mass spectrometry (first-trimester)	AGDas, AGDap in males and AGDaf, AGDac in females. Genital measurements were made by a trained study staff within a few days of birth.	Spot urine samples	Linear regression/ multivariate linear regression were used to estimate the relationship between phthalates and AGD z-score in male and female neonates with and without the hypothetical effect of hCG	MnBP, MBzP, MEHP, MEP, MiBP, MCPP, MCNP, MCOP	MEHP had a significant association with decreased AGDas in males before and after concerning the theoretical effect of hCG; higher MnBP induces shorter AGDas in males while higher MBzP induces longer AGDaf with significant difference when concerning the theoretical effect of hCG;
Bornehag 2014	196 mother-boy pairs were selected from Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA), a prospective birth cohort study	Urine samples, detected by isotope-dilution tandem mass spectrometry (first trimester, week 9-11 of pregnancy)	AGDas and AGDap, AGD was measured by two nurses (one pediatric staff nurse and one midwife) from the County Council of Värmland who were trained by research staff (20.8 ± 1.6 months, Mean ± SD)	First-trimester morning urine samples	Multiple linear regression modes were used to assess the relationship of log-transformed PMC and AGD after adjusted for adjustment for covariates (age, body size and creatinine concentrations); AGD was stratified and phthalate concentrations were compared by adjusted AGD quartile (short and long)	MBP, MEP, MBzP, oh-MMeOP, oxo-MMeOP, cx-MMeOP, MEHP, oh-MEHP, oxo-MEHP, cx-MEHP	Most of the phthalate metabolites were negatively associated with AGD before and after adjustment for covariates; all the 10 mentioned urine PMCs were much higher in short AGD group compared to long AGD group

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

Table I. Continued.

Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Definition and assessment of anogenital distance (AGD) (time of assessment)	Type of biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
Bustamante-Montes 2013	73 mothers–sons pairs, a hospital-based cohort study	Urine samples, detected by gas chromatography-mass spectroscopy (GC–MS), a modification of HPLC-MS /MS (third trimester)	AGDas, AGDap and AGDapp (the distance from the center of the anus to the posterior base of the penis), measured by 2 trained nurses (between 24 and 48 h after birth)	Third-trimester spot urine samples	Linear regression models were used to evaluate the association between prenatal MEHP and total phthalate exposure levels (μg/l), and AGDas, AGDap, AGDapp adjusted for creatinine and supine length at birth[a]	MEHP, MBP, MEP, MBzP	All three AGD measures showed inverse association trends with MEHP and "total phthalates"; shorter AGDap associated with "total phthalates" level with significance [β = −0.19mm/1μg/L (p = 0.04]; shorter AGDapp associated with MEHP [β = −0.07mm/1μg/L (p = 0.06] and "total phthalates" [β = −0.17mm/1μg/L (p = 0.09] with marginal significance
Huang 2009	65 mother–newborn pairs (33 boys, 32 girls), all participants and their fetuses were diagnosed as healthy after the chromosomes in the amniotic fluid had been evaluated	Five phthalate monoesters in amniotic fluid and urine samples from pregnant women were measured using liquid chromatography/tandem mass spectrometry (LC/MS-MS)	AGDaf in girls and AGDas in boys, measured twice by the same pediatrician and well-trained assistant (at birth)	Amniotic fluid and spot urine samples	Wilcoxon rank-sum test was used to evaluate differences between phthalates concentrations and fetal anthropometric measurements in high and low exposure group divided by Median levels; Spearman correlation coefficients was calculated to evaluate phthalates concentration and AGD	MBP, MEHP, MEP, MBzP, MMP	Association was detected between shorter AGDaf in girls and higher MBP in amniotic fluid with significance (p=0.024); and Spearman correlation coefficients showed marginally significant correlations between MBP in amniotic fluid and AGDaf in female newborns (p<0.06). Urine MBP was detected to be positively correlated with amniotic fluid MBP (R^2=0.156, p<0.05), so we may deduce: AGDaf in girls inversely associated with urinary MBP. In boys: no difference.

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

Table I. Continued.

Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Definition and assessment of anogenital distance (AGD) (time of assessment)	Type of biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
Jensen 2016	245 mother-boy pairs, eligibly selected from the Odense Child Cohort (Denmark)	Fasting spot urine samples, detected by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (median 28.7 weeks, range 26.4–30.4 weeks of gestation; second and third trimester)	AGDas, AGDap, measured three times by the same examiner, and calculated the arithmetic mean (3 months after the expected date of birth)	Fasting spot urine samples	An adjusted linear regression model was selected to evaluate the association between short AGD (AGDas) and long AGD (AGDap) in boys and quartiles of osmolality-adjusted concentrations of fasting urine phthalates metabolites	MEP, MiBP, MnBP, MBzP, ΣMBP(i + n) [b], ΣDiNPm[b], ΣDEHPm[b]	No significant dose-dependent association between any phthalate metabolites and AGDas & AGDap was found either in unadjusted or in adjusted analyses; but almost all estimates were negative, when comparing exposures above the first quartile in boys and exposures below the 25th percentile
Martino-Andrade 2016	168 mother-son pairs from TIDES	PMCs in urine samples, detected by the method of HPLC–MS⁄MS (first trimester, second trimester and third trimester)	AGDas and AGDap in boys, measured by two examiners for three times	Urine samples collected from first, second and third trimester	Multivariable linear regression models were used to determine the correlation between trimester-specific log10 SpG-adjusted concentrations of phthalate metabolites and AGDas &AGDap	MEHP, MEOHP, MEHHP, MECPP, ΣDEHP	Negative associations between log10 SpG-adjusted DEHP metabolite concentrations and both AGD measurements in T1 (data not show)
Sathyanarayana 2017	591 mother–newborn pairs selected from TIDES	Urine samples, detected by high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLCESI-MS/MS) (in early pregnancy)	AGDas and AGDap in boys, AGDaf and AGDac in girls, measured by examiners after 2-day training (shortly after birth)	Blood and urine samples	One multiple linear regression was used to explore the associations between log transformed prenatal urinary PMCs and log transformed maternal serum hormone concentrations; another multiple linear regression was used to explore the associations between log transformed prenatal hormone concentrations and AGDas, AGDap, and AGDac, and AGDaf, adjusted for several covariates[c]	MBP, MBzP, MEP, MiBP, MCNP, MEHP, MCOP, MEHHP, MEOHP, MECPP, sum DEHP	Only MCNP and MECPP correlated with lower free testosterone (FT) with statistical significance, but all DEHP metabolites were inversely related to FT. Higher maternal FT in early pregnancy was associated with a 25% lower prevalence of having a genital abnormality at birth in males, odds ratio, 0.10 (95% CI 0.01, 0.94), P<0.05. So we may deduce: higher DEHP metabolites (at least MECPP) and MCNP concentrations associated with increased prevalence of male reproductive disorders, and may induce shorter AGD

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

Table I. Continued.

Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Definition and assessment of anogenital distance (AGD) (time of assessment)	Type of biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
Suzuki 2011	Selected 111 Japanese pregnant women and their male newborns	Spot urine samples, determined by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS⁄MS) (9th to 40th week of gestation)	AGD1: from the anus to the anterior genitalia and AGD2 to the posterior genitalia, examined by medical staff 1–3 days after birth using a plastic set of digital calipers.	Spot urine	A multiple regression model was used to assess the log-transformed PMC and AGI, AGD was corrected to birth weight and defined as the anogenital index (AGI, expressed in mm⁄kg)	DEHP, MMP, MEP, MnBP, MBzP, MEHHP, MEOHP	Shorter AGI1 for DEHP with significant difference (r=-0.189, p=0.047); ΣDEHP is associated with shorter AGI1 but did not reach significance (r=-0.140, p=0.144) and inverse relationships were also seen between AGI2 and ΣDEHP
Swan 2008	140 mothers–sons pairs from Study for Future Families (SFF), a multi-center pregnancy cohort study	Urine samples, detected by isotope-dilution tandem mass spectrometry (mid-pregnancy)	AGDap in males and AGDac in females. Examined by trained physicians (first batch at 12.8 months after delivery, the second one later)	Spot urine	Divided in “shorter AGD” (lowest 25%) “intermediate AGD” and “longer AGD” (upper 25%), and compared summary statistics (mean, median and geometric mean) for metabolite concentrations	MEHP, MEHHP, MEOHP, MEP, MiBP, MMP, MBzP, MCPP, MBP (data not show)	Phthalate metabolite concentrations in “shorter” compared to “longer” AGD: serveral times higher (MEHP, MEHHP, MEOHP); higher (MEP, MiBP, MMP, MBP); lower (MCPP, MBzP)
Swan 2015	172 women (85 mother-boy pairs) included in this study were originally recruited into the first phase of the Study for Future Families (SFFI), multicenter pregnancy cohort study	Detected by the method of HPLC–MS⁄MS,	AGDas and AGDap in boys. Anthropometric measurements were conducted by medical staff under the supervision of pediatric physicians who were trained in its administration (2-36 months of age)	Blood and urine samples	In several kinds of specific PMCs, AGIs in high level group PMC compared to those in low level group PMC, ORs and 95%CIs was used to describe the difference	MBP, MEP, MBzP, MiBP	The corresponding ORs for high compared with low concentration of MBP, MEP, MBzP, and MiBP were 10.2, 4.7, 3.8, and 9.1, respectively (all p-values < 0.05)

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ethnicity and gestational age.

Table I. Continued.

Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Definition and assessment of anogenital distance (AGD) (time of assessment)	Type of biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
					In several kinds of specific PMCs, the visual inspection of mean (median) PMC levels between long and short AGI category	MBP, MEP, MBzP, MiBP	
					Divided the PMC into three categories by summary phthalate score, and compare the ORs in each concentration group	-	
Wenzel 2018	187 African American and 193 white mothers-newborns pairs	8 phthalate metabolites in urine measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), (second trimester)	AGDas, AGDap in males and AGDaf, AGDac in females, measured by one of eight trained observers in triplicate and averaged (within 48 hour of birth)	Urine specimen	Covariate-adjusted multivariable linear regression models were used to identify the correlation between PMCs and AGDas and AGDap	MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MEP, MMP, ΣDEHP, ΣDBP	Inverse associations between all measured urinary phthalates and AGDap was identified, albeit only MEHP reached significance; while statistically significant positive associations was detected for AGDas with MBP, MiBP, MBzP, and ΣDBP

In males, AGDas: the distance from the center of the anus to base of the scrotum where the skin changes rugated to smooth;
AGDap: the distance from the center of the anus to the anterior or cephalad insertion of the penis where the penile tissue meets the pubic bone;
AGDapp: the distance from the center of the anus to the posterior or caudal insertion of penis;
In females, AGDaf: the distance from the center of the anus to the base of the posterior fourchette where skin folds fuse;
AGDac: the distance from the center of the anus to the anterior tip of the clitoral hood.

[a] In this study, only a few women had exposure to MBzP, MEP and MBP above the detection levels. Thus, the statistical models only assessed the association with AGD with MEHP and total phthalates.

[b] ΣMBP(i + n), sum of MiBP and MnBP; ΣDEHPm, molar sum of DEHP metabolites expressed as excreted DEHP; ΣDiNPm, molar sum of DiNP metabolites expressed as excreted DiNP.

[c] Adjusted for covariates, including weight for length z-score, infant age at exam, study center, maternal age, maternal race/ ethnicity and gestational age.

(details provided in eligibility criteria) or urinary phthalate metabolite concentrations (PMCs). The main phthalates examined and their urinary metabolites are listed in Table II.

Study registration

This study was registered with PROSPERO describing the aims and methods in advance. The amendments specified the four target comparisons that were made between phthalate exposure and MRDs, and they discarded the initial goal of exploring AGD changes in male infants according to maternal epidemiological exposure to generic phthalates versus no exposure to phthalates. This study was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[20]

Information sources and search strategy terms

Systematic literature searches of various databases were conducted. These databases included: PubMed, Cochrane Library, Web of Science Database, Russian Science Citation Index, and the SciELO Citation Index. Initially, a search was conducted to identify articles that considered antenatal phthalate exposure and MRDs/male genital abnormalities, or any outcome of cryptorchidism, hypospadias, or AGD in humans.

Using the Boolean approach, the above-mentioned databases were searched for the following key terms in titles, keywords, and Abstracts: ("male reproductive disorders" / "male genital abnormalities" OR "cryptorchidism" OR "cryptorchism" OR "undescended test*" OR "hypospadias" OR "anogenital distance" OR "AGD") AND ("phthalat*" and its specific types). In addition, two experts in the field advised us regarding the newest relevant studies and recommended the following articles published by Swan et al.[17], Sathyanarayana et al.[21], Martino-Andrade et al.[22], and Sathyanarayana et al.[23] A manual check of the reference lists of the relevant studies and reviews that were retrieved was also conducted. As a result, three additional manuscripts were selected according to the eligibility criteria of this study.

Eligibility and exclusion criteria

Criteria for study eligibility

(1) Epidemiological studies examining generic exposure to total phthalates (with phthalates regarded as compounds rather than specific exposure to phthalate-containing products such as pesticides, cosmetics, and personal skin care products) which also provided data for an exposure group and a non-exposure group for examinations of risk of cryptorchidism, risk of hypospadias, or measurement of AGD.

(2) Clinical studies focused on exploring the relationship between risk of cryptorchidism, risk of hypospadias, or AGD and urine PMCs.

(3) Papers written in English, without restriction regarding the region studied or period of time studied, and involving human beings.

Manuscripts that satisfied criteria (1) and (3), or criteria (2) and (3), were considered eligible papers.

2.4.2 Criteria for study exclusion

(1) Animal models, reviews, guidelines, and *in vitro* or *in vivo* experimental studies.

(2) Studies addressing cellular or molecular mechanisms and other outcomes related to EDCs and MRDs (e.g., effects of hormone levels in tissues and measures of semen quality).

(3) Prenatal exposure to phthalates defined as concentrations in amniotic fluid, colostrum, or another biomarker matrix.

(4) Reports with a study design or participants that were updated in a subsequent report.

(5) Ecological studies with exposure information at the population level rather than at the individual level.

Table II. List of main specific phthalates and major urinary metabolites with abbreviations.

Phthalates name	Abbreviation	Majority usage	Urinary metabolites	Abbreviation
Di-2-ethylhexyl phthalate, Di-(2-ethylhexyl) phthalate, Diethylhexyl phthalate	DEHP	Polychlorinated vinyl (PVC), flexible plastics (toys, film packaging, medical devices, building materials, garden hoses, etc.)	Mono-2-ethylhexyl phthalate	MEHP
			Mono-(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP, oh-MEHP
			Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP, oxo-MEHP
			Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP, cx-MEPP
Di-*n*-butyl phthalate, Dibutyl phthalate	DBP	Personal care products, medications	Mono-n-butyl phthalate,	MnBP[a]
			Mono-isobutyl phthalate	MiBP[a]
Di-isobutyl phthalate	DiBP	Personal care products, medications	Mono-isobutyl phthalate	MiBP[a]
Benzylbutyl phthalate	BzBP	PVC, adhesives, sealants, car care products	Monobenzyl phthalate, Mono-benzyl phthalate	MBzP
Benzyl butyl phthalate	BBP			
Butyl benzyl phthalate	BBzP			
Di-isononyl phthalate	DINP, DiNP	PVC, flexible plastics	Mono-(carboxyoctyl) phthalate	MCOP
			Mono-isononyl phthalate	MiNP
			Mono-(4-methyl-7-hydroxyloctyl) phthalate	oh-MMeOP
			Mono-(4-methyl-7-oxo octyl) phthalate	oxo-MMeOP
			Mono-(4-methyl-7-carboxyheptyl) phthalate	cx-MMeHP
Di-isodecyl phthalate	DIDP, DiDP	PVC, flexible plastics	Mono-(carboxynonyl) phthalate	MCNP
Di-*n*-octyl phthalate	DOP, DnOP	PVC, flexible plastics	Mono-(3-carboxypropyl) phthalate	MCPP[b]
			Mono-n-octyl phthalate	MOP
Diethyl phthalate	DEP	Personal care products, fragrance	Mono-ethyl phthalate	MEP
Dimethyl phthalate	DMP		Mono-methyl phthalate	MMP

[a] sum of MnBP and MiBP is MBP.
[b] MCPP is also a minor metabolite of DBP and a non-specific metabolite of several high molecular weight phthalates(mainly refers to DEHP, BzBP, DOP, DiDP, DiNP, etc.)
AGD: anogenital distance, EDCs: endocrine disrupting chemicals, MRDs: male reproductive disorders, PMCs: phthalate metabolite concentrations.

Study selection and data extraction

Study selection

Our initial database search yielded 592 potential articles. Four additional records were identified based on recommendations made by expert authorities, and three articles were identified based on a manual review of reference lists. After 61 duplicate articles were eliminated, 538 articles were screened based on their titles and abstracts. A total of 507 reports were identified as irrelevant papers and were excluded, leaving 31 records to undergo full text screening. After a comprehensive screening of these texts, 19 manuscripts were found to conform to the eligibility criteria of this systemic review and meta-analysis. An overview of this selection process and its details are presented in Figure 1.

Data extraction

Each of the 19 articles selected was carefully reviewed and the following data were extracted: reference information; population; method and time of exposure assessment; assessment of cryptorchidism and hypospadias, measurement of AGD, and time of assessment if available; type of biomarker matrix; pivotal design and comparisons methods; specific phthalates identified; and main discoveries. For the epidemiological studies, statistical data regarding the proportion of cryptorchidism and hypospadias in exposed and non-exposed groups were recorded. These data were maintained in three electronic spreadsheets (Tables I, III–IV) for comparison of incidence of cryptorchidism, hypospadias, and AGD in relation to phthalate exposure.

Statistical analysis

It was a challenge to assess the pooled risk estimate of epidemiological exposure to generic phthalates and MRDs (mainly cryptorchidism and hypospadias). Heterogeneity of the manuscripts selected was tested by using both the Chi-squared test ($P \geq 0.1$ indicated low heterogeneity) and I^2 index statistics. When I^2 was < 50%, the Mantel-Haenszel fixed effects model was applied; otherwise, the Mantel-Haenszel random effects model was applied.[24]

In the subsequent analyses of urinary PMCs and MRDs, we analyzed possible associations between: (1) generic phthalate exposure and risk of cryptorchidism, hypospadias, and AGD; (2) specific phthalate (DEHP, DBP, benzyl butyl phthalate (BBP), etc.) exposure and incidence of generic MRDs; and (3) specific phthalate exposure and incidence of cryptorchidism, incidence of hypospadias, or AGD. Log-transformed regression of phthalate metabolite coefficients and p-values of adjusted models for AGD/anogenital index in five manuscripts are summarized in Table V.

Results

Literature search results

Figure 1 provides an overview of the screening and selection procedure used. Due to the limited number of published studies regarding human exposure to phthalates and MRDs, only 19 manuscripts met the inclusion criteria for this study. The papers that were excluded after a screening of their full text, and the corresponding reasons, are listed in Supplemental Table I. Among the 19 selected papers, 15 focused on exploring possible correlations between urinary PMCs and risk of cryptorchidism, risk of hypospadias, or measurement of AGD. The remaining four papers presented generic epidemiological studies (Wagner-Mahler et al.[25], Morales-Surez-Varela et al.[26], Nassar et al.[27], and Vrijheid et al.[28]). To date, there are no published studies which have focused on examining the risk of MRDs between mothers with undetectable urinary PMCs and those with detectable urinary PMCs.

Cryptorchidism and phthalate exposure

Two epidemiological studies compared the risk of cryptorchidism between populations exposed to generic phthalates and populations that were not exposed to phthalates. Due to the high heterogeneity between these studies, a random-

Table III. Cryptorchidism and exposure to phthalates.

Epidemiological generic exposure to phthalates							
Reference	Study type, location	Period	Population	Phthalates exposure		No phthalates exposure	
				Cryptorchidism	Normal	Cryptorchidism	Normal
Morales-Surez-Varela 2011	Danish National Birth Cohort (DNBC), Denmark	March, 1997 to November, 2002	A large number of pregnant women enrolled from DNBC	30	1441	899	43900
Wagner-Mahler 2011	prospective study, France	April, 2002 to April, 2005	Pregnant women from two main maternity wards in Nice area.	4	1	91	187

Phthalates exposure defined by measured urine phthalate metabolites concentrations							
Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Assessment of cryptorchidism (time of assessment)	Type of phthalate metabolite biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
Chevrier 2012	50 cryptorchidism and selected 149 controls nested in the EDEN and PELAGIE mother-child cohorts	Questionnaires and job-exposure matrix (JEM), the measurement methods of urinary PMCs not show. (between 6 and 30 weeks)	Examined by pediatricians or midwives during the first days after birth	Urine	The odds-ratios was calculated for undescended testis according to tertiles of urinary concentrations of phthalate metabolites to make a comparison	MEP, MBP, MiBP, MBzP,MCPP, MEHP, MEOHP, MEHHP, MECPP, MCOP, MCNP	No significant association was found between metabolites urinary levels and cryptorchidism. Only a decreased trend was found in MEP: 3rd tertile vs. 1st tertile [0.38 (0.10-1.10), p=0.06]

Table III. Continued.

Phthalates exposure defined by measured urine phthalate metabolites concentrations							
Reference	Study type, location	Period	Population	Phthalates exposure		No phthalates exposure	
				Cryptorchidism	Normal	Cryptorchidism	Normal
Sathyanarayana 2017	591 mother–newborn pairs selected from TIDES	Urine PMCs detected by HPLCESI-MS/MS (in early pregnancy)	NA	Blood and urine samples	Multiple linear regressions were used, details showed in Table 4	MBP, MBzP, MEP, MiBP, MCNP, MEHP, MCOP, MEHHP, MEOHP, MECPP, sum DEHP	Higher DEHP metabolites (at least MECPP) and MCNP concentrations associated with increased prevalence of male reproductive disorders, including cryptorchidism
Swan 2008	140 boys and 153 girls eligible pregnancy women and children selected from Study for Future Families (SFF), multi-center pregnancy cohort study	Urine samples, detected by isotope-dilution tandem mass spectrometry (mid-pregnancy)	Examined by trained physicians (first batch at 12.8 months after delivery, the second one later)	Urine	A logistic regression model controlled for age and weight percentile to see the relationship between several kinds of phthalates metabolites and cryptorchidism	Mainly DEHP metabolites: MEHP, MEHHP, MEOHP, ΣDEHP	Probably higher phthalate metabolite associated with higher risk of cryptorchidism with marginally significant difference in: MEHP (β = 1.3, P<0.05) MEHHP (β = 1.4, p=0.05) MEOHP (β = 1.4, p=0.06) ΣDEHP (β = 1.4, p=0.05)

Table IV. Hypospadias and exposure to phthalates.

Epidemiological generic exposure to phthalates							
Reference	Study type, location	Period	Population	Phthalates exposure		No phthalates exposure	
				Hypospadias	Control	Hypospadias	Control
Morales-Surez-Varela 2011	Danish National Birth Cohort (DNBC), Denmark	March, 1997 to November, 2002	A large number of pregnant women enrolled from DNBC	17	1441	227	43900
Nassar 2009	Registry-based case-control study, Australia	1980 to 2000	Hypospadias children were identified from the Western Australian Birth Defects Registry (WABDR),	48	80	1027	2209
Vrijheid 2003a	Data based on National Congenital Anomaly System (NCAS), International Agency	1980 to 1996	Hypospadias and controls (congenital anomaly) recorded from the National Congenital Anomaly System (NCAS)	341	3330	3130	32632

[a] Though control group was consist of other congenital abnormalities, we could approximately thought they were independent from each other in large-scale researches, but this must be interpreted with caution.
[b] Low-MWP comprises MEP, MBP and MiBP.

Table IV. Continued.

Phthalates exposure defined by measured urine phthalate metabolites concentrations							
Reference	Papulation, study type	Methods used for exposure assessment (time of assessment)	Assessment of hypospadias (time of assessment)	Type of biomarker matrix	Pivotal design methods and comparisons	Description of phthalates metabolite types and levels of concentrations	Main results and discoveries
Chevrier 2012	Selected 19 hypospadias and 57 controls nested in the EDEN and PELAGIE mother–child cohorts	Questionnaires and job-exposure matrix (JEM), the measurement methods of urinary PMCs not show. (between 6 and 30 weeks)	Examined by pediatricians or midwives during the first days after birth	Urine	The odds-ratios was calculated for hypospadias according to tertiles of urinary concentrations of phthalate metabolites to make a comparison	MEP, MBP, MiBP, MBzP,MCPP, MEHP, MEOHP, MEHHP, MECPP, MCOP, MCNP	No significant association was found between metabolites urinary concentration levels and hypospadias. Only a decreased trend was found in MEOHP: 3rd tertile vs. 1st tertile [0.07(0.00-1.20), p=0.09] and Low-MWP[b] [0.20 (0.02-2.50), P=0.06]
Choi 2012	Volunteer urine and plasma samples of 80 hypospadias patients and 80 controls were collected from a medical college located in Seoul (details not show)	Target compounds were detected by A GC/MS instrument consisting of gas chromatograph and mass selective detector from Agilent Technologies	NA	Urine and plasma samples	Calculated the average, maximum, and minimum concentrations, standard deviations and P-values for target compounds in the urine samples of hypospadias group and control group to make a conclusion	DBP, MBP, DEHP, MEHP	DEHP levels (P = 0.006) in the urine showed statistically significant relationships with hypospadias
Sathyanarayana 2017	591 mother–newborn pairs selected from TIDES	Urine PMCs detected by HPLCESI-MS/MS (in early pregnancy)	NA	Blood and urine samples	Multiple linear regressions were used, details showed in Table 4	MBP, MBzP, MEP, MiBP, MCNP, MEHP, MCOP, MEHHP, MEOHP, MECPP, sum DEHP	Higher DEHP metabolites (at least MECPP) and MCNP concentrations associated with increased prevalence of male reproductive disorders, including hypospadias

[a] Though control group was consist of other congenital abnormalities, we could approximately thought they were independent from each other in large-scale researches, but this must be interpreted with caution.
[b] Low-MWP comprises MEP, MBP and MiBP.

Table V. Log-transformed regression phthalate metabolite coefficients and p-value of adjusted models for anogenital distance (AGD) or anogenital index (AGI).

Reference	Design strategy and method of comparison	p< 0.05[a]			p> 0.05		
		Urine phthalate metabolite	Regression phthalate metabolite coefficients of adjusted models for AGD/ AGI	P-value	Urine phthalate metabolite	Regression phthalate metabolite coefficients of adjusted models for AGD/AGI	P-value
Log-transformed regression phthalate metabolite coefficients of adjusted models for AGD							
Barrett 2015	An interactive models was used to assess the association between PMCs and AGDas measurements to vary by maternal overall stress (lower stress and higher stress group, data not show)	molar sum ΣDEHP[b]	-1.26	0.020	MEHP	-1.14	0.300
		MEOHP	-1.44	0.010	MnBP	-0.95	0.310
		MEHHP	-1.47	0.010	MEP	-0.34	0.300
		MECPP	-0.97	0.040	MiBP	-0.52	0.360
					MCPP	0.18	0.350
					MBzP	0.15	0.600
Bornehag 2014	Multiple linear regression mode was used to assess the relationship of log-transformed PMC and AGDas after adjusted for adjustment for covariates (age, body saze and creatinine concentrations)	oh-MMeOP	-1.61	0.029	cx-MMeOP	-1.51	0.091
		oxo-MMeOP	-1.82	0.031	MEHP	-1.28	0.304
		molar sum ΣDiNP (nmol/L)	-1.69	0.047	oh-MEHP	-1.24	0.374
					oxo-MEHP	-0.77	0.576
					cx-MEHP	-0.89	0.534
					MBP	-1.41	0.351
					MEP	0.63	0.518
					MBzP	-1.66	0.088

AGD: anogenital distance (mm); AGI: anogenital index, anogenital distance divided by weight (mm/ Kg);
PMCs: phthalate metabolites concentrations;
[a] In this paper, we think p< 0.05 denotes "reach statistical significance".
[b] ΣDEHP: molar sum of four DEHP metabolites, ΣDEHP=[MEHP*(1/278)]+[MEHHP*(1/294)]+[MEOHP*(1/292)]+[MECPP*(1/308)].

Table V. Continued.

Reference	Design strategy and method of comparison	p< 0.05[a]			p> 0.05		
		Urine phthalate metabolite	Regression phthalate metabolite coefficients of adjusted models for AGD/ AGI	P-value	Urine phthalate metabolite	Regression phthalate metabolite coefficients of adjusted models for AGD/AGI	P-value
Swan 2008	A mixed model was used to control for age and weight percentile, and assess the link of logarithmically PMCs and AGD	MEHP	-3.50	0.017	MiBP	-2.96	0.097
		MEHHP	-4.98	0.002	MBzP	-0.31	0.826
		MEOHP	-5.13	0.001	MCPP	-0.97	0.591
		MEP	-2.93	0.005			
		MBP	-3.26	0.049			
		MMP	-4.00	0.053			
Log-transformed regression phthalate metabolite coefficients of adjusted models for AGI							
Suzuki 2011	A multiple regression model was used to assess the log-transformed PMC and AGI, AGD was corrected to birth weight and defined as the anogenital index (AGI, expressed in mm/kg)	MEHP	-0.25	0.011			
Swan 2015	Regression analyses of AGI on log10 PMCs controlled for age and age squared	MBP	-0.59	0.031	MBzP	-0.39	0.097
		MEP	-0.40	0.017	MCPP	-0.26	0.461
		MiBP	-0.77	0.007	MMP	-0.28	0.383
					MEHP	-0.05	0.833
					MEHHP	-0.40	0.145
					MEOHP	-0.41	0.114

AGD: anogenital distance (mm); AGI: anogenital index, anogenital distance divided by weight (mm/ Kg);
PMCs: phthalate metabolites concentrations;
[a] In this paper, we think p< 0.05 denotes "reach statistical significance".
[b] ΣDEHP: molar sum of four DEHP metabolites, ΣDEHP=[MEHP*(1/278)]+[MEHHP*(1/294)]+[MEOHP*(1/292)]+[MECPP*(1/308)].

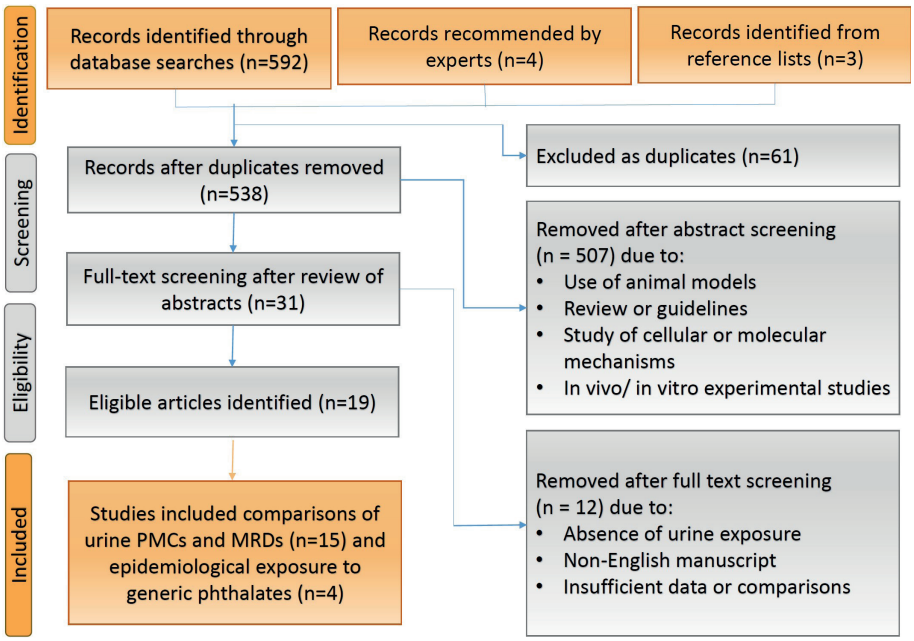

Fig. 1. Flow diagram of the systematic searches performed for various databases to identify potentially eligible publications for this study.

effect model was applied. Unfortunately, the results were not significant and a conclusion could not be made (pooled crude odds ratio (OR): 2.16; 95% confidence interval (CI): 0.30–15.45; $P = 0.44$; $I^2 = 70\%$) (Fig. 2).

Very few studies have investigated a possible association between urine PMCs and risk of cryptorchidism. However, it has been observed that the male offspring of mothers with higher PMCs of DEHP and di-isodecyl phthalate (DIDP) have a higher risk of cryptorchidism.[23,29] The design methods and findings for the phthalate-based and urine PMC-based studies are summarized in Table III.

Hypospadias and phthalate exposure

Risk of hypospadias was also examined for populations with and without exposure to phthalates in three studies.[26,28,30] Similar to the studies of cryptorchidism described above, the studies selected exhibited high heterogeneity and a random-effect model was applied. A trend towards an increased risk of having a boy affected by hypospadias was observed for mothers who were exposed to phthalates versus those who were not, although this result did not achieve statistical significance (pooled crude OR: 1.38; 95% CI: 0.93–2.04; $P = 0.11$; $I^2 = 78\%$).

A few studies have clarified a possible correlation between urine PMCs and hypospadias.[24,31] For example, the greater the exposure of pregnant mothers to DEHP and DIDP, the greater the likelihood that they will have a boy affected by hypospadias. Thus, a similar trend as that observed for cryptorchidism is also relevant for hypospadias. The design methods and findings for the phthalate-based and urine PMC-based studies are summarized in Table IV.

AGD and phthalate exposure

Eleven studies have explored a possible correlation between urine PMCs and AGD. The first study was published by Swan et al.[32] in 2005. Detailed information regarding the design methods, data comparisons, and results of all 11 studies are presented in Table IV. It was not possible to synthesize the data of these different studies to conduct a meta-analysis, since the pivotal designs of each article varied and the statistical data greatly

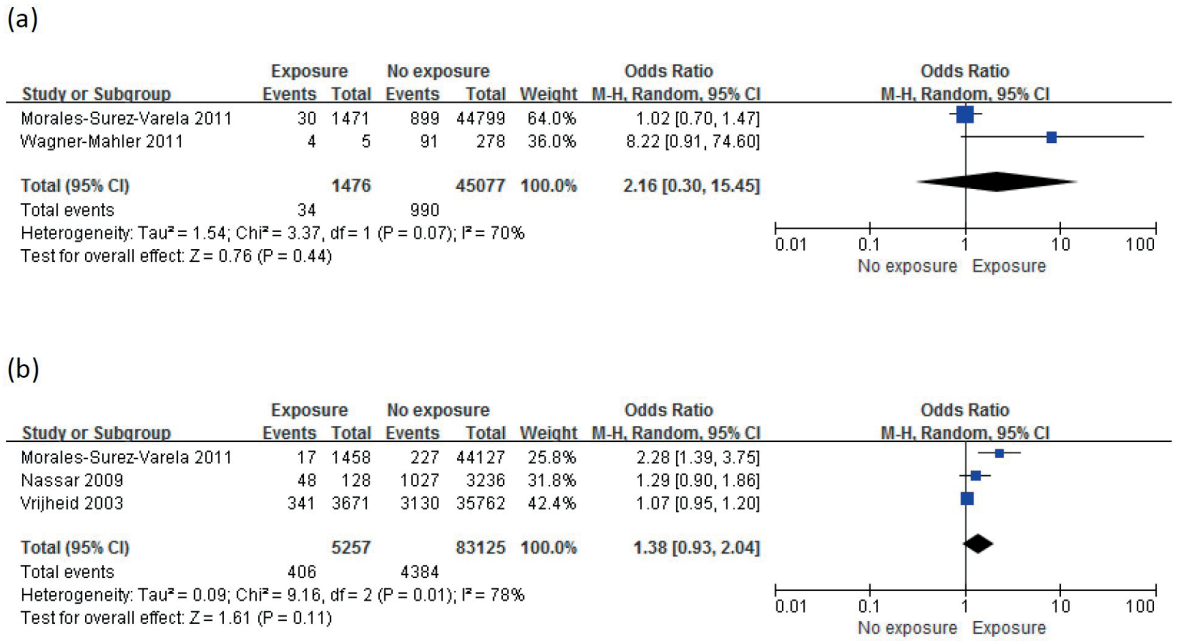

Fig. 2. Forest plot of epidemiological exposure to generic phthalates and risks of cryptorchidism (a) and hypospadias (b).

differed as well. However, we did identify an overall trend in which exposure to higher levels of phthalates was associated with a shorter AGD in humans. Moreover, a prominent association between exposure to DEHP, DBP, diethyl phthalate (DEP), or BBzP/BBP with a shorter AGD was observed. When the adjusted multiple regression model coefficients of log-transformed PMCs and AGD from 5 of the 11 articles were examined (Table V), almost all of the PMCs detected were found to be inversely associated with AGD, consistent with the overall trend observed for all 11 studies.

A graphical diagram, Figure 3, depicted these outcomes for convenience of detection of information at first sight.

Discussion

In many animal models, exposure to phthalates leads to an adverse effect on reproductive development. This phenomenon derives from the ability of phthalates to inhibit the synthesis of testosterone and disrupt androgen signaling. As a result, higher risks of cryptorchidism and hypospadias, as well as a shorter AGD, have been observed in male laboratory animals. It has been speculated that human exposure to phthalates may result in similar effects. However, it is difficult to investigate this possible correlation because specific exposure in humans is rare. Swan et al.[32] published the first human study of phthalates exposure and MRDs in 2005. Currently, there are approximately 20 manuscripts which have focused on investigations of the effects of exposure to specific phthalates on MRDs.

To clarify the adverse effects of specific compounds on human developmental health, epidemiological studies are often considered the "gold standard". However, epidemiological studies are expensive and often involve a minimum of five years from conception to results.[29] As a result, we found only two epidemiological studies which have investigated phthalate exposure and risk of cryptorchidism, and four epidemiological studies which investigated phthalate exposure and hypospadias. (Note, Ormond et al. was excluded due to insufficient data even after requests for additional data were submitted

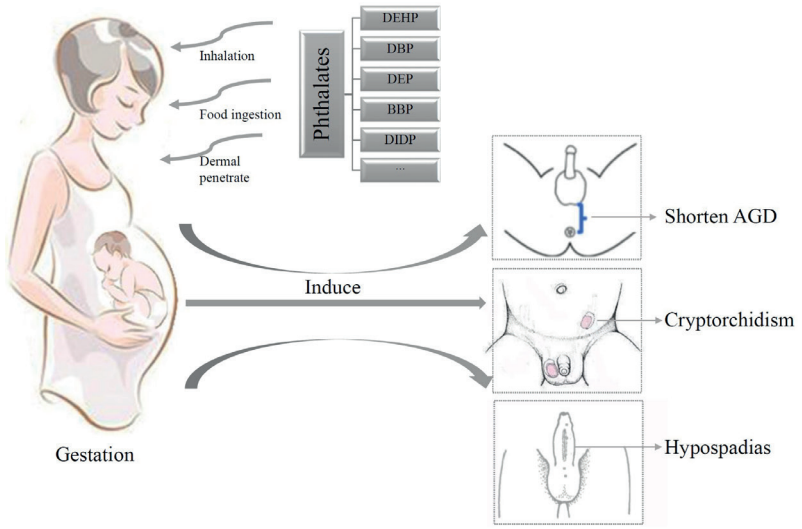

Fig. 3. Graphical diagram of effects of maternal phthalates exposure on male reproductive disorders in offsprings.

to the authors). Furthermore, we found that large-scale epidemiological studies of generic phthalate exposure were not published until after 2011. Due to this limited availability of epidemiological data, the results of our meta-analysis were rendered non-significant due to a lack of power. Moreover, our study design, which included manuscripts only written in English, may have increased the publication bias in the present study, thereby representing a limitation of this mini systematic review.

Detection and measurements of phthalate concentrations *in vitro* are complex and costly. In contrast, the excretion of high concentrations of phthalate metabolites in urine make it an optimal biomarker matrix.[33] However, a majority of phthalate metabolites may not exhibit a significant positive correlation between urine and amniotic fluid or with other biomarkers.[34] Hence, we emphasize that only urinary PMCs are regarded as indicating phthalate exposure in this paper.

Since the relationship between metabolites levels and AGD cannot be directly evaluated, the indirect methods most often used include: (1) a comparison of metabolite levels between "shorter" and "longer" AGD groups; (2) an examination of ORs of having a "shorter AGD" in individuals exposed to general or specific PMCs in different groups of higher and lower PMCs could reach statistical significance; and (3) the use of regression models to detect coefficients between log-transformed PMCs and AGD. However, the use of these various indirect methods in different studies has made it difficult to synthesize the data obtained to enhance statistical efficacy. Currently, advances in technology have allowed different phthalate metabolites to be detected in urine, and this has been applied in various studies. In this review, the majority of specific phthalates which were detected exhibited an inverse association with AGD in humans, particularly DEHP, DBP, DEP, and BBzP/BBP.[5,17,22,23,32,34-39] Furthermore, only a few studies focused on clarifying urine concentrations of phthalate metabolites and risks of cryptorchidism or hypospadias have been conducted. Thus, limited evidence is available regarding exposure to higher levels of DEHP and DIDP and increased risks of cryptorchidism and hypospadias. Furthermore, among these complex compounds, only a few have exhibited a protective effect in male genital disorders. The short-lived nature of these compounds, as well as analyses of single spot urine samples, may not effectively reflect the average exposure level to phthalates, and this

limitation existed in almost all of the included studies. In addition, all of the conclusions drawn in these studies derived from analyses of descriptive comparisons, and thus, they should be interpreted with caution.

Robust evidence has demonstrated that DEHP increases the risks of cryptorchidism and hypospadias, and shortens the AGD.[40] Di-isononyl phthalate (DINP/DiNP) has been introduced to replace DEHP, and consequently, DINP exposure has been rapidly increasing in populations worldwide. While animal data suggest that DINP may have an anti-androgen property that is similar to that of DEHP.[7,41] Bornehag et al.[5] demonstrated that urinary concentrations of DINP metabolites are also associated with a shorter AGD. Consequently, DINP exposure should be reexamined and safe replacements for harmful phthalates remains a critical need.

The effects mediated by phthalates depend on dosage, duration of action, and the stage of development for exposed individuals.[33] The primary programming period for human genital development is within the first 5–18 weeks of gestation.[23,42] During this time, phthalate metabolites can cross the placental barrier, thus, making the fetus in early pregnancy one of the most vulnerable groups to the effects of phthalate exposure. In a study conducted by Martino-Andrade et al.[22], exposure to DEHP metabolites only in the first trimester were found to be inversely associated with AGD. These findings are consistent with critical window data obtained in rodent studies[43], and they also support the biological plausibility of similar associations occurring in both humans and rodents. Therefore, avoiding exposure to phthalates during early pregnancy is the most efficient strategy for reducing the adverse effects of these compounds on male genital development in both humans and animals.

Phthalates are not covalently bound to a product matrix and they are currently used in a wide array of consumer products. In addition, their presence in the environment has made exposure to these compounds ubiquitous in daily life over the past 30 years. Consequently, phthalates have opportunities to penetrate the human body via multiple routes. Thus, nearly all human beings are exposed to phthalates, albeit at different levels. Consequently, there is not a need for more studies to evaluate the differences in risk of MRDs between mothers with and without exposure to phthalates. Rather, it would provide greater insight if well-designed studies were conducted to learn the effect of different concentration intervals of phthalate metabolites on MRDs.

The limited number of studies that have been published suggest that generic exposure to phthalates induces an adverse effect on human genital development, especially exposure to DEHP, DBP, DEP, BBP, and DIDP. Among these phthalates, DBP and DEP are most often found in personal skin care products, cosmetics, and fragrance, and this increases the exposure of pregnant women to these phthalates. As a result, public awareness of approaches to reduce phthalate exposure is important and necessary, especially during the early stages of pregnancy. Furthermore, safer substitutes that provide similar properties for plastics are greatly needed. These advances, in combination with additional well-designed and multi-center human studies, could provide valuable insight into the mechanisms mediated by phthalates and possible opportunities to prevent their adverse effects.

Multiple studies support the association between exposure to DEHP, DBP, DEP, and/or BBP and a shorter AGD. In addition, DEHP and DIDP are associated with higher risks of cryptorchidism and hypospadias. Thus, generic exposure to phthalates has an adverse effect on MRDs in both animals and humans, especially exposure to DEHP, DBP, DEP, BBP, and DIDP. Moreover, a critical time window for exposure to phthalates is during the first trimester of pregnancy, which supports the biological plausibility that similar effects are induced in both rodents and humans. However, due to the lack of significant statistical power in our

present meta-analysis, these conclusions should be interpreted with caution, and they remain to be confirmed in future well-designed studies. In the meantime, education of the public regarding phthalate exposure and the development of safer substitutes for these compounds are greatly needed.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: CY, SW, SW; data collection: CY, JL, JZ; analysis and interpretation of results: CY, JL, TZ, CL, TL; draft manuscript preparation: CY, SW, GW. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

Shengde Wu was rewarded by National Natural Science Foundation of China (NO.81873828), Chongqing Municipal Health Commission (High-level Medical Reserved Personnel Training Project of Chongqing), Innovation Program for Chongqing's Overseas Returnees (cx2019030).

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Bornehag C, Sundell J, Weschler C, et al. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. Environ Health Perspect 2004; 112: 1393-1397. https://doi.org/10.1289/ehp.7187
2. Abb M, Heinrich T, Sorkau E, Lorenz W. Phthalates in house dust. Environ Int 2009; 35: 965-970. https://doi.org/10.1016/j.envint.2009.04.007
3. Bergh C, Torgrip R, Emenius G, Ostman C. Organophosphate and phthalate esters in air and settled dust - a multi-location indoor study. Indoor Air 2011; 21: 67-76. https://doi.org/10.1111/j.1600-0668.2010.00684.x
4. Shi W, Hu X, Zhang F, et al. Occurrence of thyroid hormone activities in drinking water from eastern China: contributions of phthalate esters. Environ Sci Technol 2012; 46: 1811-1818. https://doi.org/10.1021/es202625r
5. Bornehag CG, Carlstedt F, Jonsson BAG, et al. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ Health Perspect 2015; 123: 101-107. https://doi.org/10.1289/ehp.1408163
6. Fisher J. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction 2004; 127: 305-315. https://doi.org/10.1530/rep.1.00025
7. Gray L, Ostby J, Furr J, Price M, Veeramachaneni D, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 2000; 58: 350-365. https://doi.org/10.1093/toxsci/58.2.350
8. Wilson V, Howdeshell K, Lambright C, Furr J, Earl Gray L. Differential expression of the phthalate syndrome in male Sprague-Dawley and Wistar rats after in utero DEHP exposure. Toxicol Lett 2007; 170: 177-184. https://doi.org/10.1016/j.toxlet.2007.03.004
9. Macleod D, Sharpe R, Welsh M, et al. Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl 2010; 33: 279-287. https://doi.org/10.1111/j.1365-2605.2009.01005.x
10. van den Driesche S, Scott H, MacLeod D, Fisken M, Walker M, Sharpe R. Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. Int J Androl 2011; 34: e578-586. https://doi.org/10.1111/j.1365-2605.2011.01175.x
11. Foster P. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl 2006; 29: 140-147; discussion 181-145. https://doi.org/10.1111/j.1365-2605.2005.00563.x
12. Jensen M, Nørgaard-Pedersen B, Toft G, et al. Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: temporal trends and timing of amniocentesis in pregnancy. Environ. Health Perspect 2012; 120: 897-903. https://doi.org/10.1289/ehp.1104522
13. Pierik F, Burdorf A, Nijman J, de Muinck Keizer-Schrama S, Juttmann R, Weber R. A high hypospadias rate in The Netherlands. Hum Reprod 2002; 17: 1112-1115. https://doi.org/10.1093/humrep/17.4.1112

14. Ormond G, Nieuwenhuijsen MJ, Nelson P, et al. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: case-control study. Environ Health Perspect 2009; 117: 303-307. https://doi.org/10.1289/ehp.11933

15. Braga L, Lorenzo A, Romao R. Canadian Urological Association-Pediatric Urologists of Canada (CUA-PUC) guideline for the diagnosis, management, and followup of cryptorchidism. Can Urol Assoc J 2017; 11: E251-E260. https://doi.org/10.5489/cuaj.4585

16. Barrett ES, Parlett LE, Redmon JB, Swan SH. Evidence for sexually dimorphic associations between maternal characteristics and anogenital distance, a marker of reproductive development. Am J Epidemiol 2014; 179: 57-66. https://doi.org/10.1093/aje/kwt220

17. Swan SH, Sathyanarayana S, Barrett ES, et al. First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod 2015; 30: 963-972. https://doi.org/10.1093/humrep/deu363

18. Sathyanarayana S, Grady R, Redmon JB, et al. Anogenital distance and penile width measurements in The Infant Development and the Environment Study (TIDES): methods and predictors. J Pediatr Urol 2015; 11: 76.e1-76.e766. https://doi.org/10.1016/j.jpurol.2014.11.018

19. Mendiola J, Onate-Celdran J, Samper-Mateo P, et al. Comparability and reproducibility of adult male anogenital distance measurements for two different methods. Andrology 2016; 4: 626-631. https://doi.org/10.1111/andr.12202

20. Moher D, Liberati A, Tetzlaff J, Altman D. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009; 339: b2535. https://doi.org/10.1136/bmj.b2535

21. Sathyanarayana S, Grady R, Barrett ES, et al. First trimester phthalate exposure and male newborn genital anomalies. Environ Res 2016; 151: 777-782. https://doi.org/10.1016/j.envres.2016.07.043

22. Martino-Andrade AJ, Liu F, Sathyanarayana S, et al. Timing of prenatal phthalate exposure in relation to genital endpoints in male newborns. Andrology 2016; 4: 585-593. https://doi.org/10.1111/andr.12180

23. Sathyanarayana S, Butts S, Wang C, et al. Early Prenatal Phthalate Exposure, Sex Steroid Hormones, and Birth Outcomes. J Clin Endocrinol Metab 2017; 102: 1870-1878. https://doi.org/10.1210/jc.2016-3837

24. Higgins J, Thompson S, Deeks J, Altman D. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560. https://doi.org/10.1136/bmj.327.7414.557

25. Wagner-Mahler K, Kurzenne JY, Delattre I, et al. Prospective study on the prevalence and associated risk factors of cryptorchidism in 6246 newborn boys from Nice area, France. Int J Androl 2011; 34: e499-e510. https://doi.org/10.1111/j.1365-2605.2011.01211.x

26. Morales-Suárez-Varela MM, Toft GV, Jensen MS, et al. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: a study in the Danish National Birth Cohort study. Environ Health 2011; 10: 3. https://doi.org/10.1186/1476-069X-10-3

27. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to endocrine disrupting chemicals and risk of hypospadias. Am J Epidemiol 2009;169(Suppl):S45. https://doi.org/10.1093/aje/kwp124

28. Vrijheid M, Armstrong B, Dolk H, van Tongeren M, Botting B. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med 2003; 60: 543-550. https://doi.org/10.1136/oem.60.8.543

29. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res 2008; 108: 177-184. https://doi.org/10.1016/j.envres.2008.08.007

30. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup Environ Med 2010; 67: 585-589. https://doi.org/10.1136/oem.2009.048272

31. Choi H, Kim J, Im Y, Lee S, Kim Y. The association between some endocrine disruptors and hypospadias in biological samples. J Environ Sci Health A Tox Hazard Subst Environ Eng 2012; 47: 2173-2179. https://doi.org/10.1080/10934529.2012.680387

32. Swan SH, Main KM, Liu F, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 2005; 113: 1056-1061. https://doi.org/10.1289/ehp.8100

33. Bajkin I, Bjelica A, Icin T, Dobrić V, Zavisić BK, Stojanoska MM. Effects of phthalic acid esters on fetal health. Med Pregl 2014; 67: 172-175. https://doi.org/10.2298/MPNS1406172B

34. Huang PC, Kuo PL, Chou YY, Lin SJ, Lee CC. Association between prenatal exposure to phthalates and the health of newborns. Environ Int 2009; 35: 14-20. https://doi.org/10.1016/j.envint.2008.05.012

35. Adibi JJ, Lee MK, Naimi AI, et al. Human chorionic gonadotropin partially mediates phthalate association with male and female anogenital distance. J Clin Endocrinol Metab 2015; 100: E1216-E1224. https://doi.org/10.1210/jc.2015-2370

36. Bustamante-Montes LP, Hernández-Valero MA, Flores-Pimentel D, et al. Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns. J Dev Orig Health Dis 2013; 4: 300-306. https://doi.org/10.1017/S2040174413000172

37. Jensen TK, Frederiksen H, Kyhl HB, et al. Prenatal exposure to phthalates and anogenital distance in male infants from a low-exposed Danish cohort (2010-2012). Environ Health Perspect 2016; 124: 1107-1113. https://doi.org/10.1289/ehp.1509870

38. Suzuki Y, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H. Foetal exposure to phthalate esters and anogenital distance in male newborns. Int J Androl 2012; 35: 236-244. https://doi.org/10.1111/j.1365-2605.2011.01190.x

39. Wenzel AG, Bloom MS, Butts CD, et al. Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls. Environ Int 2018; 110: 61-70. https://doi.org/10.1016/j.envint.2017.10.007

40. Dorman DC, Chiu W, Hales BF, et al. Systematic reviews and meta-analyses of human and animal evidence of prenatal diethylhexyl phthalate exposure and changes in male anogenital distance. J Toxicol Environ Health B Crit Rev 2018; 21: 207-226. https://doi.org/10.1080/10937404.2018.1505354

41. Boberg J, Christiansen S, Axelstad M, et al. Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol 2011; 31: 200-209. https://doi.org/10.1016/j.reprotox.2010.11.001

42. Rey R, Picard JY. Embryology and endocrinology of genital development. Baillieres Clin Endocrinol Metab 1998; 12: 17-33. https://doi.org/10.1016/s0950-351x(98)80427-8

43. Welsh M, Saunders PT, Fisken M, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest 2008; 118: 1479-1490. https://doi.org/10.1172/JCI34241