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Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans

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ABSTRACT

After briefly discussing human exposure to phthalates—diesters of 1,2-benzenedicarboxylic acid (phthalic acid)—this article first presents recent findings from the Study for Future Families, a multi-center pregnancy study in which the human analogue of the phthalate syndrome was first identified. This is one of an increasing number of studies that have investigated human endpoints in relation to environmental exposure to these ubiquitous compounds. This literature, which includes a range of human health endpoints following prenatal, neonatal, childhood, and adult exposures, is then summarized. At least one significant association has been reported for urinary metabolites of di-*n*-butyl phthalate (DBP), butylbenzyl phthalate (BzBP), diethyl phthalate (DEP), and di-isononyl phthalate (DINP) and for three of the urinary metabolites of di(2-ethylhexyl) phthalate (DEHP). Many of the findings reported in humans—most of which have been in males—are consistent with the anti-androgenic action that has been demonstrated for several phthalates. Replication of the results described here and further mechanistic studies are needed to strengthen links between phthalates and adverse health outcomes.

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1. Background

Diesters of 1,2-benzenedicarboxylic acid (phthalic acid), commonly referred to as phthalates, are man-made chemicals widely used in industry and commerce. This group of man-made chemicals has a wide spectrum of industrial applications, and these chemicals appear, ultimately, in a wide range of consumer products, as well as in food processing and in medical applications.

Di(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl phthalate (DBP), and benzylbutyl phthalate (BzBP), and di-isononyl phthalate (DINP) have been shown to disrupt reproductive tract development in male rodents in an anti-androgenic manner (Parks et al., 2000). Based on recent data from dose–response studies with DEHP, DBP, and BzBP, Howdeshell et al. (2008b) reported that DBP, DEHP and BzBP were equipotent at reducing fetal testicular testosterone production. Most adverse effects examined in animal studies have been reproductive, and findings are predominantly in males, but it should be noted that females have been less well studied. In addition, a number of non-reproductive endpoints (including some in females) have been reported in this literature, often at high doses, including; hepatic and renal effects (DBP), hepatocellular carcinoma (DEHP), anovulation, and decreased fetal growth (DEHP) (Hauser and Calafat, 2005).

This review summarizes recent data on human exposure to phthalates at environmental levels and associated health endpoints in humans. Though the number of phthalates is large, many have not yet been examined in humans. After briefly discussing sources and routes of environmental exposure to these phthalates, I will present recent data on male genital endpoints from the Study for Future Families (SFF), a pregnancy cohort study in which the human analogue of the phthalate syndrome was first identified. I will then briefly review the growing body of epidemiologic studies that have investigated other health outcomes that have been associated in the literature with exposure to these ubiquitous compounds.

2. Exposure to phthalates

2.1. Sources of phthalates in the environment

Phthalates have been measured in residential indoor environments in both house dust and indoor air (Rudel et al., 2003). They have also been measured in foods, milk, and drinking water. However, the relative contribution from the various sources and routes of exposure to phthalates is unknown (Wormuth et al., 2006).

High-molecular-weight phthalates, such as DEHP, are primarily used as plasticizers in the manufacture of polyvinyl chloride

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(PVC), which is used extensively in consumer products, flooring, and wall coverings, as well as food contact applications, and medical devices (ATSDR, 2002). Exposure to DEHP can occur in the workplace (e.g. in the manufacture of DEHP or DEHP containing products, or workers using these products), during consumer use of these products, or through environmental media (food, air, water, dust). The inclusion of DEHP in products to which children are exposed (e.g. toys, bath books), particularly those used by young children, and medical devices such as intravenous tubing and blood and nutrient bags are of particular concern because of the vulnerability of these populations.

Lower molecular weight phthalates, including DBP and diethyl phthalate (DEP), are used as solvents and plasticizers for cellulose acetate, in making lacquers, varnishes, personal-care products (e.g. perfumes, lotions, and cosmetics), and coatings (ATSDR, 1995, 2001), including those used in making timed-release pharmaceuticals (Hauser et al., 2004a). DEP (urinary metabolite MEP) is found in population samples at levels that are often an order of magnitude higher than those of DEHP and DBP (CDC, 2003, 2005). DEP has been found in a large number of cosmetics and personal care products, and, in adults (Duty et al., 2005a) and young children (Sathyanarayana et al., 2008), linked to use of such products.

2.2. Routes of exposure to phthalates

Humans are exposed to phthalates by multiple routes, and the most likely route varies by phthalate. Exposures can be oral (e.g. DEHP via phthalate-contaminated food, water and other liquids and in children through mouthing of toys and teething) or dermal (e.g. DEP, via cosmetics and other personal care products). Exposure can also be via inhalation; phthalates volatilize from PVC, nail polish, hair spray, and other phthalate-containing products, and parenteral (e.g. DEHP via tubing used in neonatal intensive care nurseries) (FDA, 2001). In contrast, in almost all rodent studies to date, exposure is oral. Therefore, these rodent studies may not reflect toxicity of phthalates to humans who are exposed via other routes. For example, human exposure to DEP is primarily dermal and, secondarily via inhalation. While most animal studies on DEP and MEP do not find reproductive toxicity (ATSDR, 1995), several of the human studies that have examined this phthalate have found significant adverse associations (see Tables 4 and 5). This apparent inconsistency may be the result, at least in part, of differing exposure routes.

2.3. Assessing human exposure to phthalates

Because of the silent and pervasive nature of environmental phthalate exposure, traditional epidemiological methods of exposure assessment (questionnaires, medical records, etc.) are of limited usefulness in determining individual exposure. Instead, biomarkers of exposure are preferred. Urine is the preferred matrix for phthalate determination in humans (Calafat and McKee, 2006). Because of rapid metabolism, (except in infants younger than 12 weeks) urinary metabolite levels are typically higher, and, therefore, more precisely measured than levels of the parent compound found in other media. Further, by measuring metabolites, the risk of accidental contamination during collection, storage, and analysis is greatly reduced. Phthalates or their metabolites have also been measured in many body fluids and matrices, including; urine, serum, saliva, seminal fluid, breast milk, amniotic fluid, meconium, and even placenta.

While exposures during fetal development are more likely to result in profound and permanent alterations than postnatal exposures, conducting such studies in human populations is

challenging. First, in order to estimate prenatal exposure reliably, it is necessary to assay biological samples obtained and archived during pregnancy. Second, such studies require reliable assessment of outcomes in the offspring of those pregnancies. While this is relatively easy for perinatal outcomes, in order to examine outcomes occurring in later life—such as impaired semen quality and infertility—it is necessary to follow the pregnancy cohort for decades. Thus, logistic and financial difficulties account, at least in part, for the limited number of such studies, and the complete absence of any examination of adult effects of prenatal exposure to phthalates. On the other hand, assessing effects of postnatal or adult exposure in relation to outcomes measured concurrent with, or soon after, sample collection is more feasible.

The recent availability of sensitive, specific, and economical assays make such measures practicable for epidemiological studies (Silva et al., 2007). Moreover, international quality control programs for assays of phthalate metabolites in human samples help to maximize comparability of results across laboratories internationally.

Phthalates are rapidly metabolized in the body, with elimination half-lives of less than 24 h (Koch et al., 2005). Several studies have examined the within-person variability of phthalate metabolites. Hoppin et al. (Hoppin et al., 2002) found that the Pearson correlation coefficient in concentration in women's samples taken two days apart ranged from 0.5 for mBzP to 0.8 for mBP and concluded that despite their short half-lives, exposure may be sufficiently stable to assign an exposure level based on a single sample. Similarly, Hauser et al. (Hauser et al., 2004b) concluded that despite substantial day-to-day and month-to-month variability in men's individual urinary phthalate metabolite levels, a single urine sample was moderately predictive of each subject's exposure over three months. A recent review by Teitelbaum et al. (Teitelbaum et al., 2008) of sources of variability in biomarkers of environmental exposures in minority children, including phthalates, confirms that a single spot urine sample is sufficiently representative of exposure over a six-month period to warrant its use as an exposure estimate in epidemiological studies.

The Centers for Disease Control and Prevention (CDC) has published data on levels of phthalate metabolites in a large population-based sample of the US population over six years of age (CDC, 2003, 2005). These data demonstrate the ubiquitous nature of these chemicals and variation in concentration by ethnicity, sex, and age. A European group (Wormuth et al., 2006) has modeled estimated phthalate levels in a European population, including infants, and estimates of exposure to DEHP and DBP in infants are about one order of magnitude higher than those in adults. A recent analysis of phthalate metabolite concentration in 163 infants (2–36 months) shows that all are exposed to one or more phthalates and 80% to seven or more. Moreover, infant levels of MEP and MBP are directly related to the number of baby care products (lotions, creams, and powders) applied by their mothers (Sathyanarayana et al., 2008).

3. Health outcomes associated with human prenatal exposure to phthalates

There were few studies conducted of human health outcomes associated with phthalate exposure prior to 2000. However, by 2000, the availability of sensitive, specific, and affordable bioassays made biomarkers feasible for use in epidemiological studies. Moreover, the growing rodent literature provided convincing data on the reproductive toxicity of several commonly used phthalates (Howdeshell et al., 2008a).

We begin this discussion with the one study, to date, to have examined health outcomes in humans in relation to prenatal

phthalate exposure, and the rodent data that motivated that study. We then summarize the literature on human health endpoints that have been associated with environmental exposure to phthalates.

3.1. Evidence for the “phthalate syndrome” in humans

Over the past ten years, increasingly detailed rodent studies have demonstrated that several phthalates (most notably DEHP, DBP, and BzBP), when administered at the critical window for the development of the reproductive tract, disrupt the androgen-signaling pathway in males (Akingbemi et al., 2004; Borch et al., 2004; Foster, 2006; Gray, 2000). Recently Lee et al. demonstrated the anti-androgenic effects of seven phthalates (DEHP and its metabolite, MEHP, DBP, BzBP as well as DINP, DIDP, DnHP) by the Hershberger assay in castrated male SD rats (Lee and Koo, 2007). This anti-androgenic action results in interference with the normal cascade of androgen-dependent outcomes, most notably shortened anogenital distance (AGD). AGD, which in newborn rodent pups is the distance from the anus to the genital tubercle, is approximately twice as long in males as females. AGD is a sensitive marker of fetal exposure to anti-androgens, including phthalates, vinclozilin, and flutamide. Prior to 2005, only a single human study had evaluated AGD in males (Salazar-Martinez et al., 2004), and two other studies had evaluated AGD in female infants (Phillip et al., 1996; Callegari et al., 1987). These studies demonstrated the sexual dimorphism of AGD in humans, consistent with rodent studies. However, these studies did not examine the relationship of AGD to chemical exposure.

Several animal studies report that DBP, DEHP, and BzBP induce a marked reduction in fetal testosterone (produced by Leydig cells) and insulin-like growth factor-3 (Insl-3), resulting in a syndrome of male reproductive abnormalities, which, in addition to shortened AGD, include hypospadias, cryptorchidism, and malformations of the epididymis, vas deferens, seminal vesicles, and prostate; together they comprise the “phthalate syndrome” (Welsh et al., 2008; Foster, 2006).

While a variety of anti-androgens alter AGD in rodents, most are androgen receptor (AR) antagonists. The syndrome induced by the AR-antagonists differs from that induced by the phthalates, which inhibit the synthesis of fetal testosterone and Insl-3 (Wilson et al., 2004, 2008). A variety of potentially anti-androgenic substances including procymidone (P), linuron (L), flutamide (F), and *p,p'*-DDE were tested. P, L, and *p,p'*-DDE produced F-like profiles, that included shortened AGD but were distinct from those produced by DBP and DEHP (Gray, 2000). Thus, detection of shortened AGD appears to signal prenatal anti-androgen exposure, but knowledge of the entire profile of genital dysmorphism is necessary to pinpoint a particular etiologic agent.

3.2. Phthalates in relation to genital development in human infants

3.2.1. Study population

The SFF is a multi-center pregnancy cohort study in which we collected and stored urine and serum samples obtained mid-pregnancy. The availability of these samples, allowed us to link prenatal phthalate exposure to outcomes in the children born to couples enrolled in SFF. Therefore, in 2000, we initiated the Study of Phthalates in Pregnant Women and Children (PPWC) in order to examine AGD and other genital parameters in human infants in relation to their mother's phthalate exposure. We hypothesized that a human analogue of the “phthalate syndrome” would be found in male infants. Initial results from that study, previously published (Swan et al., 2005) are updated here.

Study methods for SFF are described in detail elsewhere (Swan et al., 2003). Briefly, couples whose pregnancy was not medically assisted were eligible to participate unless the woman or her partner was less than 18 years, either partner did not read and speak Spanish or English, or the father was unavailable or unknown. All participants completed a questionnaire and most gave blood samples. Maternal and paternal urine collection was added to the protocol in the second study year. If the participant had agreed to be recontacted (and 85% had), we invited the mother to take part in a follow-up study. The family was eligible for PPWC if the baby was 2–36 months of age when contacted, the family lived within 50 miles of the clinic, and could attend at least one study visit. Fifty-one percent of SFF subjects participated in this follow-up study. We present data on results of the physical examination in 140 boys and 153 girls from Minnesota, MN, Columbia, MO and Los Angeles, CA, and results of the phthalate analysis on the approximately 75% of these for whom both maternal urine samples and physical examination data were available. Human subject committees at all participating institutions approved both SFF and PPWC and all subjects signed informed consents for each study.

3.2.2. Infant measurements

At the first postnatal study visit (mean 12.8 months after delivery) a detailed examination of breast and genitals, developed for this study, was conducted under the supervision of pediatric physicians who were trained in its administration. The male examination included a description of the testes and scrotum, location of testes, measurement of the penis, and AGD. All measurements (AGD and penile size) were made with a precision calipers. In males, AGD is the distance (in mm) from the center of the anus to the cephalad (towards the head) base of the penis, while female AGD is the distance (in mm) from the center of the anus to the cephalad base of the clitoris.

Examiners also evaluated testicular descent, which was classified as “incomplete” if one or both testicles were found not to be “normal” or “normal retractile”. In addition, penile length and width were obtained. Neither the pediatric physicians nor the support staff had any knowledge of the mother's phthalate concentration.

3.2.3. Phthalate measurements

Because SFF was not, initially, designed to examine biomarkers of prenatal exposure (but rather semen quality across four areas of the US) urine collection was begun only midway through the study. There were 106 mother-son pairs with both prenatal phthalate concentration and boy's genital measurements. Phthalate metabolite concentrations were assayed in urine samples provided during pregnancy (mean 28.6 week of pregnancy, as measured from the last menstrual period), using a sensitive method that involves the enzymatic deconjugation of the phthalate metabolites from their glucuronidated form, automated on-line solid-phase extraction, separation with high performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry (Silva et al., 2007).

3.2.4. Statistical analysis

Because AGD varies with age and weight, it is necessary to control for these variables in analysis. In the 2005 analysis, guided by practice in rodent studies (Hotchkiss and Vandenberg, 2005), we divided AGD by weight to create the anogenital index. While this is common practice in rodent studies, for our babies of varying ages, this method did completely eliminate confounding by weight. Therefore, in this updated analysis, we used weight percentile, a quantity that is largely independent of weight and

when included as a covariate in the model eliminates residual confounding by weight. Therefore, for each infant, the expected weight for age (weight percentile) was determined by sex-specific estimates of weight percentiles in the US population (<http://www.cdc.gov/growthcharts/>).

The 2005 publication included AGD and phthalate results for the first visit of 85 boys. In the current analysis, we include 106 boys for whom we had measured mother's prenatal phthalate concentration, 68 of whom had AGD measured at two visits. Because of the inclusion of multiple observations for these boys, data were analyzed using a mixed model, controlling for age and weight percentile.

We also conducted an analysis of metabolite levels in relation to a categorical (three-valued) measure of AGD. For this purpose, we compared each boy's observed AGD to that expected based on his weight and age. We then divided boys into three categories, using the difference between the observed and expected AGD, which we call the residual AGD. Boys whose residual AGD fell in the lowest 25% were classified as having "shorter AGD", those in the upper 25% as having "longer AGD" and others as having "intermediate AGD". We then compared summary statistics (mean, median, and geometric mean) for metabolite concentrations among these three groups of boys.

To examine joint exposure to multiple phthalates, we created a "summary phthalate score" that reflects combined exposure to the five phthalate metabolites associated with male AGD (MEHP, MEOHP, MEHHP, MBP, and MEP). To construct the summary score, first the distribution of these five metabolites was divided into quartiles. The score was increased by 1 point for each additional quartile in which the metabolite fell, so each metabolite can contribute 0, 1, 2, or 3 points to the summary score. If the concentration of all five metabolites fell in the lowest quartile, the summary score for that woman was 0. The maximum score of 15 was assigned when all five metabolites fell in the upper quartile. We called the summary exposure "low" if the score was 0–3, "high" if the score was 12–15, and medium otherwise.

3.2.5. Results

In boys AGD averaged 70.4 mm (SD 10.8 mm), while in girls, AGD averaged 47.3 mm (SD 7.1 mm). Male AGD was, therefore, 49% longer on average than female AGD, with a standard deviation that was greater. Thus, in humans, as in rodents, this measure is strongly sexually dimorphic, although, as can be seen in Fig. 1, there is considerable overlap of the AGD distributions in males and females in this population. Salazar-Martinez et al. reported an AGD in 42 newborn males that was twice that in 45 newborn females (Salazar-Martinez et al., 2004). In that population, the distribution of AGD in male and female were nearly non-overlapping, perhaps the result of minimal prenatal exposure to anti-androgens.

Tables 1–3 contain results of new analyses of phthalate metabolite levels in relation to AGD, penile width, and testicular descent.

Table 1 contains the estimate of the beta-coefficient for each metabolite (logarithmically transformed to normalize distributions) and its *p*-value in regression models that controlled for age and weight percentile. In this data set, urinary concentrations of five metabolites were significantly and inversely related to AGD, MEP, MBP, and the three DEHP metabolites measured (MEHP, MEOHP, and MEHHP). These results differ somewhat from our 2005 analysis, particularly in relation to DEHP metabolites, which in the prior analysis were either not significant (MEHP) or of borderline significance (MEOHP and MEHHP). In addition, MBZP, which was of borderline significance in the earlier analysis (*p* = 0.055) is no longer associated with AGD.

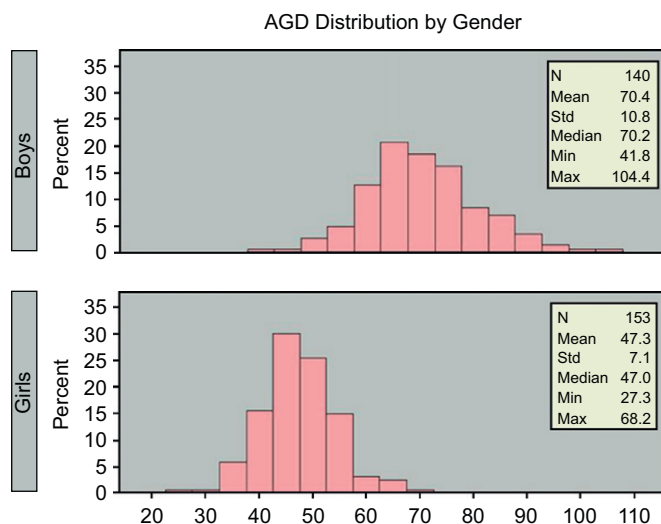


Fig. 1. Distribution of anogenital distance (mm) in boys and girls.

Table 1
AGD in relation to phthalate metabolite concentration^a

Monoester metabolite	Coefficient of log ₁₀ metabolite concentration	<i>p</i> -value of coefficient	% change in AGD for IQ ^b increase in metabolite concentration (%)
MEHP	−3.503	0.017	−4.4
MEHHP	−4.977	0.002	−3.9
MEOHP	−5.126	0.001	−4.5
MEP	−2.934	0.005	−4.0
MBP	−3.255	0.049	−3.2
MIBP	−2.9562	0.097	−3.5
MMP	−4.0002	0.053	−3.6
MBZP	−0.3129	0.826	−0.4
MCP	−0.9650	0.591	−1.0

^a Using a mixed model controlling for age and weight percentile.

^b Estimated change in AGD for an increase in phthalate metabolite concentration from 25th percentile to 75th percentile.

As seen in Table 2, for several metabolites, most notably the DEHP metabolites (MEHP, MEHHP, and MEOHP) and MEP, the mean metabolite concentration was several times greater among boys with "shorter" compared to "longer" AGD.

Residual AGD was correlated with both penile width and length: correlations 0.31 and 0.16 (*p* = 0.0003 and 0.059, respectively). In a mixed model controlling for age and weight percentile, penile width (but not length) was significantly associated with concentration of the sum of DEHP metabolites (\sum DEHP) and MEHP. No other metabolites were associated with penile size (Table 3).

Of boys for whom both testicular location and phthalate concentrations were available (*N* = 119), 12 had incomplete testicular descent (10.1%). As can be seen in Table 3, higher DEHP metabolite concentration was significantly associated with a greater probability of incomplete testicular descent. No other phthalate metabolites were associated with testicular descent.

We then examined joint exposure to the five phthalate metabolites associated with AGD using the summary phthalate score. The median score was 9 with a range of 0–15. In this population, the summary phthalate was more predictive of AGD category than individual metabolite concentrations. Of the 29 boys with a "shorter" AGD, none were born to a mother with a low summary phthalate score. Conversely, of the 28 boys with a "longer" AGD, only one was born to a mother with a high

Table 2
AGD and phthalate metabolite concentrations: categorical analysis

Monoester metabolite	Anogenital distance category		
	Longer (N = 26)	Intermediate (N = 51)	Shorter (N = 29)
	Mean/median/ geometric mean (ng/mL)	Mean/median/geometric mean (ng/mL)	Mean/median/geometric mean (ng/mL)
MEHP	4.4/2.3/2.5	6.4/2.9/3.1	12.3/6.2/5.8
MEHHP	12.0/8.2/8.0	18.3/10.0/9.8	37.9/19.8/20.9
MEOHP	10.1/7.3/7.0	16.2/11.7/8.7	33.9/21.3/19.3
MEP	191.9/80.5/78.5	599.2/92.7/110.2	910.3/277.3/252.1
MBP	21.5/11.4/12.6	19.2/14.2/12.1	39.9/26.4/24.0
MiBP	2.8/1.5/1.8	4.0/2.3/2.4	6.1/3.4/3.8
MMP	1.6/0.7/1.1	2.3/0.7/1.4	2.7/1.4/1.6
MBzP	22.0/8.3/8.8	20.5/5.6/8.3	16.8/10.8/10.5
MCP	8.1/1.7/1.8	2.9/1.6/1.7	2.8/2.5/2.2

^aUsing a mixed model controlling for age and weight percentile.

Table 3
Penile width, testicular descent and DEHP metabolite concentration: regression analyses

Monoester metabolite	Coefficient of log ₁₀ metabolite concentration		p-value of coefficient	
	Penile width ^a	Testicular descent ^b	Penile width ^a	Testicular descent ^b
MEHP	-0.782	-1.258	0.005	0.048
MEHHP	-0.530	-1.417	0.080	0.054
MEOHP	-0.466	-1.350	0.120	0.064
ΣDEHP	-0.570	-1.447	0.072	0.052

^a Using a mixed model controlling for age and weight percentile.

^b Using a logistic regression model controlling for age and weight percentile.

summary phthalate score (p -value <0.00001). While numbers in this study were too small to distinguish dose additivity from effect additivity, this finding is consistent with the rodent studies demonstrating dose additivity of multiple phthalate exposure.

3.2.6. Conclusions from PPWC

In 2005, we published initial findings on boy's AGD in relation to the concentration of mothers' urinary phthalate metabolites (Swan et al., 2005). The updated data presented here (Tables 1–3) differ from the 2005 findings in three ways. First, the 2005 analysis included a smaller data set (85 boys with one visit per boy). Second, the statistical methods employed in the current analysis have been improved, as explained above. Third, the current analysis examines phthalate concentration in relation to both AGD and two other endpoints consistent with the phthalate syndrome (e.g. penile width and testicular descent). Thus, in this recent analysis, we see largely results consistent with and extending those previously published (Swan et al., 2005). In the current analysis, we find significant associations between AGD and the three metabolites of DEHP, as well as their sum, which were not seen in the prior analysis. We also see a direct relationship between DEHP metabolites (most notably MEHP) and penile width, which were not seen previously. Additionally, the MEHP metabolites were significantly and inversely related to testicular descent. These findings warrant current concerns that low dose phthalate exposures affect several markers of human male genital development.

3.3. Summary of health outcomes in infants and children

Turning to other health outcomes in infants and children, published studies that have examined these outcomes in associa-

tion with prenatal, perinatal, and early childhood phthalate exposure are contained in Table 4.

3.3.1. Phthalates and infant hormones

Main et al. (Main et al., 2006) measured phthalate monoesters in breast milk samples collected 1–3 months after birth. Gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin-b were measured in babies' serum samples collected at three months. All phthalate monoesters were found in breast milk. MEP and MBP were positively correlated with SHBG ($r = 0.323$, $p = 0.002$ and $r = 0.272$, $p = 0.01$, respectively); MMP, MEP, and MBP with the ratio of LH to free testosterone ($r = 0.21$ – 0.323 , $p = 0.002$ – 0.044) and MiNP with luteinizing hormone ($r = 0.243$, $p = 0.019$). MBP was negatively correlated with free testosterone ($r = -0.22$, $p = 0.033$).

The results on male genital development from PPWC and these data on neonatal male hormone concentrations in relation to phthalate exposure are consistent and together suggest that the phthalate syndrome originally identified in rodents (Gray et al., 2006) may also be occurring in male babies whose mothers were exposed to higher levels of one or more phthalates during pregnancy.

3.3.2. Phthalates and gestational age at delivery

Only one published study to date has examined gestational age in relation to phthalate exposure. Latini et al. (Latini et al., 2003) measured serum DEHP and MEHP in the cord blood of 84 infants born in Brindisi, Italy, and examined concentration in relation to gestational age (which was not defined). DEHP and MEHP were each present in 65 of 84 (77.4%) of the examined samples. Infants for whom MEHP was detectable in cord blood were categorized as "MEHP-positive" (with a similar definition for

Table 4
Health outcomes in infants and children associated with phthalate concentration in biological or environmental samples

System	Timing of exposure	Sex	Outcome	Phthalate or metabolite (measured in urine unless otherwise noted)	Reference	
Reproductive	Prenatal (mean age 12.6 months at exam)	Males/Females	Shorter gestational age at birth	MEHP (in cord blood)	Latini et al. (2003)	
		Males	Shorter anogenital distance	MEHP, MEOHP, MEHHP, MEP, MBP		Swan et al. (2005) and Tables 1–3
	Lactation (mean age 3 months)	Males	Reduced penile size Incomplete testicular descent Increased SHBG	MEHP MEHP, MEHHP, MEOHP MEP, MBP	Main et al. (2006)	
Respiratory, allergy and asthma	Early childhood	Females	Increased LH/free T Increased LH	MMP, MEP, MBP MiNP	Colon et al. (2000)	
	Childhood	Males/Females	Decreased free T	MBP		
				Premature thelarche	DEHP (in serum)	Bornehag et al. (2004)
				Rhinitis and eczema Asthma Wheezing, rhinitis and eczema	BzBP (in house dust) DEHP (in house dust) DEHP (in house dust)	Kolarik et al. (2008)

Table 5
Health outcomes in adults associated with phthalate concentration in biological samples

System	Sex	Outcome	Phthalate or metabolite (measured in urine unless otherwise noted)	Reference
Reproductive	Males	Increased sperm DNA damage	MEP, MEHP	Hauser et al. (2007) Duty et al. (2003b) Duty et al. (2003a), Hauser et al. (2006)
		Increased sperm DNA damage	MEP	
		Decreased sperm motility	MBP	
		Decreased sperm concentration	MBP, MBzP	Duty et al. (2003a), Hauser et al. (2006)
	Males	Decreased sperm morphology	DEHP in semen samples	Zhang et al. (2006)
	Males	Decreased free T and increased LH/free T	MBP	Pan et al. (2006)
Respiratory and immune (incl. allergy and asthma)	Males	Decreased FSH	MBzP	Duty et al. (2005a, b)
	Males	Decreased motility	MEP	Jonsson et al. (2005)
	Males	Reduced LH	MEP, MBP	Hoppin et al. (2004)
Metabolic	Males	Decreased pulmonary function	MEP, MBP	Hoppin et al. (2004)
Thyroid	Males	Increased waist circumference	MBzP, MEHHP, MEOHP, MEP	Stahlhut et al. (2007)
		Increased insulin resistance	MBP, MBzP, MEP	
		Thyroid (decreased T3 and T4)	MEHP	Meeker et al. (2007)

DEHP). MEHP-positive newborns showed a significantly lower gestational age compared with MEHP-negative infants (38.16 ± 2.34 vs. 39.35 ± 1.35 ; $t = -2.163$, $p = 0.033$). No associations were found for DEHP, and no significant relations were observed between either DEHP or MEHP and birth weight.

3.3.3. Phthalates and premature thelarche

Colon et al. (Colon et al., 2000) compared serum levels of phthalates in 41 Puerto Rican girls (median age 20 months) with premature thelarche (premature breast development) and 35 pediatric controls (median age 46 months). The largest differences between cases and controls were between serum DEHP concentration in cases and controls; 450 and 70 ng/ml, respectively ($p < 0.05$). There are concerns regarding several aspects of the study design (McKee and Toxicology Research Task, 2004) including potential sample contamination, the large age difference between cases and controls, and the fact that exposure was measured concurrent with diagnosis.

3.3.4. Phthalates in relation to respiratory function, asthma, and allergy

A number of studies have examined respiratory function, asthma, and allergy in children in relation to the use of PVC-containing products in the home or phthalates in house dust

(Bornehag et al., 2004; Jaakkola et al., 1999, 2000), although none has used biomarkers to define exposure. Two cross-sectional studies found associations between allergic symptoms and house dust levels of BzBP and DEHP (Bornehag et al., 2004; Kolarik et al., 2008). In the future, biomarker studies will provide more precise measures of exposure and clarify the strength of these relationships.

3.4. Summary of health outcomes in adults

Studies that have examined health outcomes in adults in relation to recent phthalate exposure are summarized in Table 5.

3.4.1. Phthalates in relation to semen quality and sex hormones in males

Duty, Hauser, and colleagues published a series of studies that examined urinary phthalate levels and semen characteristics, sperm DNA damage, and serum reproductive hormones in men attending an infertility clinic (Duty et al., 2003a, b, 2005a, b; Hauser et al., 2004a, b). There were dose-response relationships of MBP with low sperm concentration (odds ratio per quartile, adjusted for age, abstinence time, and smoking status (1.0, 3.1, 2.5, 3.3; p for trend = 0.04) and motility (1.0, 1.5, 1.5, 1.8; p for trend = 0.04). There was suggestive evidence of an association

between the highest MBzP quartile and low sperm concentration (1.0, 1.1, 1.1, 1.9; p for trend = 0.13). There were no relationships of MEP, MMP or the DEHP metabolites with these semen parameters. These results differed from those reported by Jonsson et al. (Jonsson et al., 2005), who did not observe any significant associations between semen quality and phthalate exposure in a study of young healthy military recruits in Sweden. However, several differences were noted between the US and Swedish studies (Hauser et al., 2006), including the age and fertility status of the study populations. A small study in Shanghai (Zhang et al., 2006) examined concentration of three phthalates (DEHP, DBP, and DEP) in semen samples. No associations were seen with sperm concentration. All three phthalates were positively associated with sample liquefaction time, and DEHP was significantly correlated to “rate of malformation” (presumably impaired morphology).

In the US population studied by Hauser and Duty, there was evidence of sperm DNA damage as assessed by the neutral comet assay, in association with higher exposure to MEP and MEHP (Hauser et al., 2007; Duty et al., 2003a, b). Duty et al. (Duty et al., 2005a, b) also examined the relationships between urinary levels of phthalate metabolites and serum levels of FSH, LH, SHBG, testosterone, and inhibin-B in this population. They found that after adjusting for age, body mass index, and time of day that the blood sample was drawn, only MBzP was associated with a decrease in FSH. In addition, there was an increase of borderline significance in inhibin-B associated with MBP. No other phthalate metabolites were associated with hormones in this population. However, Pan et al. (Pan et al., 2006) reported a significant decrease in free testosterone, and increased LH to free testosterone ratio in association with MBP concentration in an occupationally exposed cohort.

3.4.2. Phthalates in relation to respiratory function, metabolism, and thyroid function in adults

Other than studies of male reproduction function, there are few human studies of phthalate effects in adults. Only one study in adults examined respiratory function. Hoppin et al. examined the association between phthalate exposure and four pulmonary function parameters in the Third National Health and Nutrition Examination Survey (NHANES, 1988–1994). After controlling for race, age, height, body mass index, and smoking, there was an inverse association between MBP and three measures of pulmonary function (FVC, FEV₁, PEF), and higher MEP was associated with lower FVC and FEV₁ values in men. No consistent associations were seen in females or with other phthalate metabolites (Hoppin et al., 2004).

Only a single study to date has examined markers of metabolic syndrome in relation to phthalate exposure. Stahlhut et al. (Stahlhut et al., 2007) investigated phthalate exposure and its associations with abdominal obesity and insulin resistance in adult US male participants in the NHANES 1999–2002. They modeled six phthalate metabolites as predictors of waist circumference and HOMA (a measure of insulin resistance) using multiple linear regression, adjusted for age, race/ethnicity, fat and total calorie consumption, physical activity level, serum cotinine, and urine creatinine. Four metabolites were significantly associated with increased waist circumference (MBzP, MEHHP, MEOHP, and MEP) and three with increased HOMA (MBP, MBzP, and MEP).

Meeker et al. (Meeker et al., 2007) examined urinary concentrations of MEHP and other phthalate monoester metabolites in 408 male partners in subfertile couples and concluded that Urinary MEHP concentrations may be associated with altered free T4 and/or total T3 levels in adult men. They saw an inverse

association between MEHP urinary concentrations and free T4 and T3 serum levels. The inverse relationship between MEHP and free T4 remained when adjusting for oxidative metabolite concentrations, which simultaneously demonstrated a suggestive positive association with free T4.

4. Conclusions

In the relatively short time (7–8 years) that sensitive biomarkers for assaying human phthalate exposure in humans have been available, a rapidly growing literature has demonstrated that human exposure is ubiquitous, and evidence for significant impacts on human health is mounting. As seen in this review, most studies in both the toxicological and human literature have reported associations with prenatal and postnatal phthalate exposure in males. This may be a consequence of the anti-androgenic mode of action of the most toxic of these chemicals. In addition (as was learned from the delay in detecting effects of diethylstilbestrol in females), alteration in the female genital tract are hidden and may be missed until puberty or adulthood.

Epidemiological studies may be the “gold standard” for assessing risks to human health, but these are expensive (costing upwards of one-million US dollars per study) and slow (typically a minimum of five years from conception of a human health study to results). Moreover, epidemiology is limited in its ability to draw causal inferences. Therefore, it must be informed by, and closely linked to, animal studies in which mechanism and causality can be demonstrated. While several links between rodent and human studies have been made in this area, as discussed above, these are limited by several discrepancies between human and animal testing.

Animal testing of phthalates has been, for the most part, conducted at doses far higher than those present in the ambient human environment. If dose responses are nonlinear, as has been demonstrated for other environmental chemicals, this testing strategy will not protect human health (vom Saal and Hughes, 2005). In addition, until recently phthalates were tested singly, while humans are exposed to multiple phthalates simultaneously. Recent rodent data suggest that exposure to multiple phthalates at low doses conveys risk in a dose additive manner (Gray et al., 2006; Howdeshell et al., 2008a, b). This suggests that the risk from a mixture of phthalates (or phthalates and other anti-androgens), whether acting by similar mechanisms or not, cannot be accurately assessed studying one chemical at a time.

The same may be true of risks from multiple exposure routes. For example, most toxicological testing of phthalate is via oral exposure. However, humans are exposed to phthalates by a multiplicity of routes. For DEP, and its metabolite MEP, human exposure is predominantly dermal. For example, Janjua et al. (Janjua et al., 2007) recently demonstrated systemic dermal uptake of MEP and MBP in male volunteers by measuring these in serum and urine following whole body topical application of cream containing these compounds. Whether differences in exposure route can account for differences in the toxicology of this phthalate in humans and rodents is not clear.

Because of these important differences in route, dose level, and multiplicity of agents, it is often unclear how to interpret findings across species. For these reasons, if chemical risk assessment is to be relevant to human exposure, future assessments should incorporate more environmentally relevant scenarios, including mixtures.

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