A Review of Male Reproductive Endpoints Associated with Exposure to Common Phthalates

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Table of Contents

I. Abstract 3
II. Introduction 3
III. Cumulative Risk Assessment and MOAs 4
IV. Background 6
V. Biomarkers and Exposure 9
VI. Critical Window 10
VII. Anogenital Distance 11
VIII. Nipple Retention 14
IX. Hypospadias 15
X. Cryptorchidism 16
XI. Sperm Quality 17
XII. Conclusion 19
XIII. Appendix 22
XIV. References
Abstract

Phthalates are a class of chemical compounds used in many industrial, military, medical, and consumer products as solvents or plasticizers to make plastics more malleable. Phthalates are ubiquitous and numerous studies have demonstrated the pervasiveness of human phthalate exposure at all stages of life, making phthalates a subject of great concern to toxicologists, pharmacologists, and health specialists. This concern led the European Union and United States to ban the use of multiple phthalates in children’s toys and the European Union to take restrictions one step further by banning phthalates in cosmetics (NRC, 2008). An association between phthalates and male reproductive endpoints, which are collectively termed the phthalate syndrome, has been shown. Phthalate syndrome is the range of effects on the reproductive system in male animals including sperm quality, cryptorchidism, hypospadias, and reduced anogenital distance. Recent studies have also shown that the reproductive effects from phthalate exposure occur at much lower doses than previously predicted and researchers now believe that the cumulative effects of multiple phthalates are greater than previously predicted. This literature review will assess the major risks to male reproduction associated with phthalate exposure in a dose-response and cumulative risk assessment approach. Further review of phthalate syndrome studies will lead to better understanding of the role phthalates play in reproductive effects and ultimately, help influence regulations on phthalate use.

Introduction

Phthalates are diesters of phthalic acid that are widely used in an eclectic variety of industrial processes and consumer and industrial products that humans routinely use such as cosmetics, toys, pharmaceuticals, food packaging, vinyl gloves, and garden hoses. Phthalates are
used as plasticizers that are added to plastic polymers to make them more malleable; as fixatives and solvents in cosmetics, perfumes, detergents, and insecticides; as adhesives in food packaging; and many more utilities. Phthalates have become so ubiquitous that scientists now believe humans are exposed to multiple phthalates simultaneously via ingestion, inhalation, dermal absorption, parenteral routes, and prenatally throughout the course of our lives from the neonatal stage to senescence (NRC, 2008).

Medical reports from the past 50 years have noted an increased incidence of male reproductive health issues including a decline in sperm counts, and increased incidence of cryptorchidism, hypospadias, and other urogenital malformations. Previous studies have shown that synthetic chemicals in the environment, such as pesticides, cause negative reproductive outcomes through endocrine mechanisms and naturally-occurring estrogens in the environment cause infertility (Gray et al., 2000). Given the results of these studies and medical reports, it was suggested that in utero exposure to environmental estrogens may be causing the increase in malformations, decrease in sperm count, and the altered sex-ratio of human populations (Sharpe and Skakkebaek, 1993). Progestins, the synthetic version of progesterone, are known androgen (male sex hormone) antagonists that disrupt masculinization during sexual differentiation (Schardelin, 1993). And progesterone is a steroid hormone that plays a critical role in the production of other steroid hormones, such as estrogens and other sex hormones. This evidence supported the hypothesis that environmental estrogens exposed to fetuses in utero cause negative male reproductive health outcomes, leading many researchers explore the relationship between androgen antagonists, also called anti-androgens, and male reproductive endpoints (Figure 1). Numerous studies have demonstrated the anti-androgenic properties of phthalates (Gray et al., 1999a; Mlychreest et al., 1999; Parks et al., 2000). In response to these finding, researchers have
begun looking into the association between these male reproductive endpoints, collectively termed the phthalate syndrome, and phthalates.

**Cumulative Risk Assessment and MOAs**

Cumulative risk refers to the combined threats via multiple biologic pathways and exposure routes posed by biological, chemical and physical stressors to which people are exposed for varied durations. Cumulative risk assessment is therefore a means of organizing and analyzing cumulative risk data in order to distinguish and quantify the collective adverse effects on humans and/or the environment. This review addresses the risk of exposure to multiple phthalates leading to common adverse outcomes therefore a cumulative risk approach is taken, though some individual phthalates are noted periodically. A recent study demonstrated that the combination of nine phthalates (DEHP, DBP, DCHP, BBP, DPENP, DIBP, DIHEPP, and DHEPP) followed the additivity assumption that individual phthalates exert effects without altering the effects of other phthalates in relation to fetal androgen synthesis (Hannas *et al.*, 2012). This means that the effects of a mixed solution can be predicted accurately when concentrations of each phthalate are known and that, ultimately, cumulative risk models can be developed to predict health outcomes (CHAP, 2014). Cumulative risk assessment also allows researchers to determine if phthalates which show no effects individually could combine to cause negative health outcomes. Howdeshell *et al.* (2008) studied testosterone synthesis suppression after exposure to five phthalates and found that though each phthalate was not expected to cause significant effects at the dose used, they combined to cause statistically significant testosterone suppression (NRC, 2008). Phthalates in this review were selected because they are classified by EPA under phthalate chemical class and because they contain the phthalate ester side chain. In
this review the terms endpoints and common adverse outcome both refer to the negative health outcomes caused by the chemicals of interest, phthalates.

Mode of action and mechanism of action both describe the biologic pathway of a chemical that leads to a specific endpoint, or health outcome. While these terms are commonly used interchangeably, they are actually differentiated by the level of detail they describe in the pathway. The mode of action defines key events occurring on the pathway and the mechanism of action defines the molecular processes of the pathway. Traditionally cumulative risk assessment has been approached by looking at chemicals with common mechanisms of action, but focusing cumulative risk assessment on the endpoint, or health outcomes, is more effective because many pathways can lead to the same endpoint therefore focusing on one pathway alone limits the scope of the risk assessment. While the mechanisms of action for phthalate toxicity are still being developed, key phthalate-susceptible pathways in the male reproductive tract development have been identified (Figure 1).

Background

Phthalates are diesters of benzenedicarboxylic acid with varying sizes of ester side chains. Generally phthalates containing 3 – 6 carbons in the ester side chain have the most potent effects on the male reproductive system (NRC, 2008). Phthalates are classified into size groups by the number of carbons in their side ester chains; long contain ≥ 7 carbons in the side chain, medium contain 4 - 6 carbons in the side chain, and short contain ≤ 3 carbons in the side chain. Phthalates are chemical plasticizers in polymers and solvents that are added to increase the flexibility of the plastic. However, because the phthalates do not chemically bind to the polymer matrix, they readily migrate or off-gas into surrounding environments, particularly when the
material endures high-temperatures. Once leached into the environment, these phthalates are taken up by crops and animals, thereby entering the human food supply (NTP, 2002). Humans are exposed to phthalates via ingestion, inhalation, dermal absorption, parenteral routes, and prenatally (NRC, 2008).

Exposure to phthalates is considered widespread because phthalates are found in a wide variety of products ranging from medical devices, food packaging, cosmetics and construction materials (Table 1). Phthalates with high molecular weight are most commonly used as plasticizers in polyvinyl chloride (PVC) while low molecular weight phthalates are solvents and plasticizers for cellulose acetate which is used in lacquers, cosmetics, and medication coatings (Table 1). Phthalates are so pervasive that exposures to multiple phthalates often occur concurrently in all life stages from pre-natal to adult (NRC, 2008). Numerous studies address the prenatal effects because phthalates have been shown to cross the placenta, have been detected in amniotic fluid, and because the fetus is the most sensitive life stage (NRC, 2008). Most data on phthalate exposure are obtained through in vivo and in vitro laboratory testing using rats because they are more sensitive to phthalates than other laboratory species such as mice, hamsters, and guinea pigs (Gray et al., 1982).

The cumulative adverse outcomes in males are collectively called the phthalate syndrome which is characterized by malformations of the epididymis, vas deferens, seminal vesicles, prostate, hypospadias (urethra malformation in the penis), cryptorchidism (undescended testes), and by retention of nipples, infertility, decreased sperm count and motility, and reduced anogenital distance (Mylchreest et al., 1999; NRC, 2008). Phthalate syndrome effects are caused by disturbances in androgens, or male sex hormones, such as testosterone, the primary androgen. Testosterone and its more potent metabolite dihydrotestosterone (DHT) determine the male
phenotype during fetal sexual differentiation. Testosterone mediates the differentiation of epididymis, vas deferens, and seminal vesicles, collectively called the Wolffian structures, and DHT mediates the masculinization of external genitalia and the prostate (Gray et al., 2000).

Schaffer et al. first observed male reproductive malformations following exposure to di-(2-ethylhexyl) phthalate in 1945, bringing phthalates into the spotlight of male reproductive toxicity testing. Since then, phthalates have become the most studied of all testicular toxicants, though relatively few human and epidemiological studies are available (Boekelheide, 2004) due to financial limitations, ethical concerns, and logistical complications of procuring a sufficient number of participants. Some phthalate syndrome effects, such as infertility and sperm dysfunction, cannot be detected until later in life and therefore subjects must be observed over multiple decades which can be very difficult and expensive to manage. Since 2000, more epidemiological studies have been conducted because, prior to then, affordable, sensitive and specific bioassays were not readily available to test biomarkers for chemical toxicity (Swan, 2008).

Multiple hypotheses were proposed over the years to explain the mechanism by which phthalates cause male reproductive injury such as changes in zinc-dependent activity, hormonal levels, metabolic function, and Sertoli-germ cell adhesion. These hypotheses all propose models in which the Sertoli cell, the “nurse” cell responsible for spermatogenesis, is the cell target for toxicity because studies had shown that phthalate exposure caused early histopathological changes to Sertoli cells but no changes to occurred in endpoints that are typically highly responsive to hormones. These studies, however, were all performed using a single high dose of phthalates administered to test animals or in-vitro assays, cells cultures, but later research revealed that phthalates have effects at respectively lower doses. After Jobling et al (1995)
revealed that BBP and DBP are weakly estrogenic, more researchers began to explore the androgenic implications of phthalate exposure and it was determined that while Sertoli cells were the target cells in adolescents and adults, Leydig cells, responsible for testosterone production, are the cell targets of toxicity during natal and post-natal development (Boekelheide, 2004). The discovery of the androgen-disrupting properties of phthalates led to a deeper understanding the mechanisms by which phthalates cause male reproductive malformations.

**Biomarkers and Exposure**

Humans are exposed to phthalates via inhalation, orally, dermally and parenterally by routes other than the GI tract, such as intravenously. Phthalates volatize from PVC and other products are inhaled, phthalates in cosmetics and personal-care products are absorbed through the skin, phthalate-contaminated food and beverages (from plastic food wraps and containers) and toys containing phthalates enter the body orally, and phthalates in medical tubing enter the body intravenously (Swan, 2008). Biomarkers, or molecular events linking exposure to outcome, for phthalate toxicity are most commonly urinary polar metabolites because their concentration is anywhere from 5-20 times greater than in lipid-rich tissues and because polar molecules are very efficiently excreted through urine (NRC, 2008). Blood serum, breast-milk, saliva, amniotic fluid, meconium, placenta, and seminal fluids can also be used to determine phthalate exposure (Swan, 2008), though are much less common than urine. Metabolites, as opposed to the parent compounds, are measured because phthalates have a half-life of less than 24 hours and therefore metabolize quickly (Koch et al., 2005). Also, measuring metabolites reduces potential contamination from collecting, storing and analyzing samples because phthalates are ubiquitous and could leach into the sample from multiple sources, but metabolites are only created within the body. The National Health and Nutrition Examination Survey (NHANES) is a program of
studies within the Center for Disease Control (CDC) that “assess the health and nutritional status of adults and children in the United States [by combining] interviews and physical examinations” (CDC). NHANES studies on phthalates demonstrated urinary metabolite concentrations were higher in children aged 6 – 11 than in adults and adolescents and studies performed by other major labs supported these findings (NRC, 2008). Researchers are still trying to determine if these higher concentrations in children are due to differences in exposure, metabolism, or both because there numerous factors that affect exposure of children compared to adults. It has been determined that fetuses and infants can be exposed to multiple phthalates (Sathyanarayana, 2008; Adibi, 2009; Wolff, 2008) which complicates the models of exposure. Children tend to ingest more calories per kilogram of body weight, higher fat-content foods (NRC, 2008), and more dairy products than adolescents and adults and fats, oils, cheese, cream have consistently high phthalate concentrations (Serrano, 2014). Infants and young children are also more likely to experience higher exposure orally due to mouthing or teething on toys and other plastic objects where phthalates are present and by inhalation from indoor air because they have higher specific respiratory rates than adults do. These factors and findings have led the European Union and the United States to restrict phthalate concentrations in children’s toys (NRC, 2008). Neonates receiving medical treatments like transfusions are considered the group of highest exposure because medical tubing and devices made from PVCs containing phthalates can leach 10-20 mg of phthalates per day and often medications use phthalates as a coating (Loff et al., 2000).

Critical Window

Phthalate reproductive toxicity and risk assessment progressed substantially when the teratology protocol was changed to reflect the critical developmental window when sexual differentiation occurs. Early teratogenic studies, studies addressing abnormalities of
physiological development, dosed pregnant rats during gestation days (GD) 6-15, the critical window for organ development and current teratology protocol calls for examination of the fetus right before birth. However, the critical window for sexual differentiation in rats occurs between GD 12-21 and the effects of phthalate syndrome cannot be diagnosed at that time without a full histopathologic examination which current protocol does not require. With this understanding, researchers studying phthalate toxicity have altered the protocol to include the critical window for phthalate toxicity exposure and require that subjects are examined post-natally when phthalate syndrome effects can be observed. The critical window for male reproductive malformations can vary slightly between phthalates so researchers are beginning to identify critical windows for individual phthalates. For example, Carruthers et al. (2005) suggest the critical window for DBP is GD 16 – 18. In human fetuses, the male reproductive critical window falls between gestational week 8 and 12 (Sharpe et al.)

**Anogenital Distance**

Lee and Koo (2007) demonstrated that DEHP, MEHP, DBP, BBP, DINP, DIDP, and DHP have anti-androgenic effects in male rats and other researchers (Foster et al. 2006; Grey et al. 2000; Borch et al. 2004) suggest that phthalates, specifically DBP, DEHP, and BBP, disrupt androgen-signaling pathways when rodents are exposed during the reproductive tract critical window of development. This disruption in androgen activity is what causes the various effects of phthalate syndrome, the most notable of which is reduced anogenital distance. Multiple human studies have shown that reduced anogenital distance is a relevant biomarker for phthalate syndrome in humans because it is associated with hypospadias (Hseih et al., 2008), poor relative semen quality (Mendiola, 2011), and infertility (Eisenberg, 2011). Anogenital distance in males is measured from the anus to the base of the scrotum and, in females, from the anus to the
frenulum of labia minor, the tissue that joins the labia minora at the base of the vagina. Anogenital distance is more commonly measured in males because it is roughly twice as long in males as in females (Figure 3). Anogenital distance is a convenient measure of anti-androgen exposure during the critical window because it is non-invasive and can be measured over the entire lifetime of the subject (Sharpe et al.) and because anogenital development is sensitive to anti-androgenic substances (Swan, 2008).

In a teratology study by Carruthers et al. (2005), pregnant Sprague-Dawley rats were dosed with Di-n-butyl phthalate (DBP) prenatally for a 2 day period on gestational days 14 and 15, 15 and 16, 16 and 17, 17 and 18, 18 and 19, and 19 and 20. Various male reproductive endpoints were measured including anogenital distance, nipple retention, and the weights of testes, epididymis, prostate glands, seminal vesicles, liver, and kidneys. The anogenital distance of the male pups was measured at birth, at postnatal day 13, and at postnatal day 90 when male pups were necropsied. Anogenital distance showed statistically significant permanent reductions in size in male pups dosed with DBP prenatally at gestation days 15 and 16 or 18 and 19. This study provides evidence that exposure to di-butyl phthalate has substantial negative effects on fetal male reproductive development causing a permanently reduced anogenital distance and also identifies the critical window for male reproductive development at gestational days 16-18 based on the exposure days that caused the most effects for each endpoint. Identifying the critical window for abnormal reproductive development is vital to fully investigating the reproductive effects of phthalates.

Another prospective cohort study followed 85 mother-son pairs (Swan et al., 2005) that participated in the Study for Future Families, a national pregnancy cohort study conducted in prenatal clinics in California, Minnesota, Missouri, and Iowa, and agreed to be in a follow-up
study in which the boys’ genitals, including anogenital distance, were measured and analyzed. Researchers calculated the anogenital index (AGI), a weight-normalized index of anogenital distance, by dividing the anogenital distance by the subject’s weight (mm/kg), and then developed a summary phthalate score from those results to assess the effects from exposure to multiple metabolites. The summary phthalate score divides the anogenital index scores into quartiles and boys falling in the 25th percentile were categorized as having a short anogenital index, boys between 25th and 75th as intermediate, and boys above the 75th percentile as having a long anogenital index.

The phthalate summary score is directly related to a short anogenital index; 9 out of 10 boys with high phthalate score had a short anogenital index and only 1 of 11 boys with a low phthalate score had a short anogenital index. These results showed that high urinary concentrations of metabolites MBP, MiBP, MEP, and MBzP are associated with a decreased anogenital distance in human males. Researchers also observed that the 85 mothers tested were exposed to metabolites at every detectable level and that those levels of phthalate metabolites were highly correlated to one another; meaning that if one metabolite was present in high concentration levels, the other metabolites were also present in high concentrations. This high correlation between metabolite levels substantiates the argument that cumulative risk assessment is the best approach for assessing reproductive phthalate toxicity. While this study had a relatively small sample size, results are consistent with those from the larger NHANES study. Comparison of the results confirm that these four urinary metabolites (MBP, MiBP, MEP, and MBzP) are prevalent in females in the U.S. and that levels of these metabolites in the 85 mothers whose sons have a short anogenital distance were not abnormally higher than in other women across the U.S. This means that the women in the Study for Future Families had normal urinary
metabolite levels relative to other U.S. women and therefore, this study provides strong evidence that these metabolites have adverse outcomes on the reproductive development anogenital distance in males (Swan et al., 2005).

Numerous studies aside from the above mentioned have shown reduced anogenital distance in males. Liu et al. (2005) found significant reduced anogenital distance after exposure to DBP, BBP, DPP, and DEHP. Mylchreest et al. (1999) found that exposure to DBP during the critical window for male sexual differentiation at gestation days 12 - 21 resulted in a dose-dependent decrease in anogenital distance in males with a 9% decrease at 250 mg/kg/day and a 24% decrease at 500 mg/kg/day. Anogenital distance was reduced by about 30% in male rat pups 2 days old, but not in female pups.

**Nipple Retention**

Carruthers et al. (2005) dosed pregnant Sprague-Dawley rats with Di-n-butyl phthalate (DBP) prenatally on gestational days (GD) 14 and 15, 15 and 16, 16 and 17, 17 and 18, 18 and 19, and 19 and 20. The presence or absence of areolas was recorded at postnatal day (PND) 13 and again at PND 90 when males were necropsied. By PND 13, pups who were exposed to DBP on GD 15 and 16 or GD 18 and 19 presented areolas, but by PND 90 the areolas recessed. Only pups exposed on GD 16 and 17 retained the areolas until necropsy. These results suggest that the critical window of exposure for abnormal reproductive development occurs from GD 16 – 18 and that 2-day DBP exposure damages male reproductive tract development (Carruthers, 2005). These nipple retention results corroborate the results of anogenital distance from the same study, as mentioned above.
Doses of BBP, DEHP, and DINP administered immediately before and after birth significantly increased nipple retention in male pups (Gray et al., 2000). Typically male rats have no nipples and female rats have 12. In this study, however, most male pups dosed with BBP, DEHP, and DINP retained anywhere from 1 – 14 permanent nipples, a gross malformation in rats particularly because unlike humans, male rats do not retain nipples after the fetal stage (Gray et al., 2000).

Hypospadias

Hypospadias is a congenital condition affecting human males in which the opening of the urethra underneath the penis instead of the tip. In males with hypospadias, the urethra forms abnormally during gestation weeks 8–14 and can occur anywhere ranging from below the tip of the penis to the scrotum (Figure 2). This malformation causes difficulty urinating and could lead to problems performing sexual intercourse later in life. Hypospadias, along with cryptorchidism and other genital malformations, is associated with reduced anogenital distance in boys (Hsieh et al., 2008). Several studies link male reproductive tract malformations, including hypospadias, to in utero exposure of male rat pups to high doses of DEHP, DBP, BBP and DINP (Foster, 2006; Gray et al., 2006; Mylchreest et al., 2000).

One study (Ormond et al., 2009) assessed the risk of hypospadias associated with maternal occupational exposure to endocrine-disrupting chemical such as phthalates compared with the use of folate supplementation during pregnancy and vegetarianism. The mothers held jobs as hairdressers, beauty therapists, research chemists, line operators, pharmaceutical operators, electrical assemblers, factory assistants, off of which expose them to elevated levels of phthalates. The study found significantly higher risk of hypospadias was found in boys of
mothers occupationally exposed to phthalates than in boys whose mothers did not experience occupational exposure (Ormond et al., 2009).

**Cryptorchidism**

Cryptorchidism, a condition in which one or both testicles fail to descend into the scrotum, is among the most common human malformations that affects 3% of males at birth and 1% of males after their first year. Cryptorchidism can lead to fertility impairment, testicular cancer, testicular torsion (when a testicle rotates, cutting off blood flow to the scrotum) and groin hernia (Kolon et al., 2014). Phthalates act as anti-androgens in the body and inhibit insulin-like hormone 3 (insl3), an androgen involved in testicular descent (Carruthers et al., 2005). In utero exposure to phthalates decreases the fetal testes’ insl3 mRNA concentration which substantiates the hypothesis that phthalates inhibit insl3 expression during sexual differentiation and ultimately leads to cryptorchidism (Wilson et al., 2004).

Phthalates DEHP and BBP caused undescended testes in male pups dosed with 750 mg/kg/day from gestation day 14 to postnatal day 3 (Gray et al., 2000). And a higher MEHP concentration was significantly and inversely associated with testicular descent meaning the higher the MEHP, the lower the probability of complete descent (Swan, 2008). Another study found that 30% male rats dosed with 500 mg/kg/day of DBP from gestational day 3 to postnatal day 30 experienced cryptorchidism of one or both testes.

A particularly fascinating study found MBP not only inhibited testicular descent, but also induced testicular ascent into the abdominal cavity (Imajima et al., 1997). Pregnant rats were exposed to mono-n-butyl phthalate (MBP) by gavage from gestational days 15 – 18 and given cesarean sections on gestational day 20 at which point the pups were necropsied. Dissection of
the male pups showed significantly higher abdominal ascent of the testes in MBP-dosed rats than that in those of the control rats. And 22 out of 26 (about 84.6%) male pups dosed with MBP exhibited cryptorchidism of one or both testes at 30 to 40 days old, while zero control rats exhibited cryptorchidism. Sertoli cells secrete Müllerian-inhibiting substance which mediates the transabdominal phase and therefore this study suggests that prenatal MBP exposure can impair Sertoli that in turn cause testicular ascension into the abdominal cavity and cryptorchidism (Imajima et al., 1997).

**Sperm Quality**

A paper published in 1992 analyzed international data on semen quality in young men and suggested that concentration and count had declined over a the past 50 years, sparking heated debates among and a series of semen quality studies (Carleson et al. 1992). These studies were mostly conducted using national data from sperm banks and donor registries and yielded contradicting results but a later updated analysis substantiated Carleson et al. claim (Swan et al., 2008). Concurrently, more evidence of a global increase in the incidence of testicular cancer, which is associated with decreased sperm quality, was published. These findings are concerning because of the implications they have for human fecundity, or reproductive success. A large cross-sectional study involving 4867 young men of median age 19 was undertaken in Denmark from 1996 – 2010. Participants’ semen was analyzed for semen volume, sperm concentration, sperm count, sperm motility and sperm morphology. While the results demonstrated a positive trend in sperm concentration and count, only 23%, or one in four men, had ideal semen quality. Though the trend was increasing for two semen parameters, the proportion of good to poor semen quality is concerning. With 3 in 4 males showing inferior sperm quality, it is likely that fertility rates will decrease and the need for fertility treatments will increase. These alarming
results have prompted researchers to look into the mechanisms by which semen quality declines in males.

A series of studies examined urinary phthalate levels and semen parameters, sperm DNA damage, and blood hormone levels of men attending an infertility clinic (Duty et al., 2005; Duty et al., 2003a; Duty et al., 2003b; Hauser et al., 2004). These studies demonstrated the dose–response relationship of MBP with low sperm concentration and motility and suggested an association between the upper quartile (with the highest MBzP concentrations) and low sperm concentration, which is defined as the odds ratio per quartile for the sample population and was adjusted for age, period of abstinence, and smoking status because smoking cigarettes is associated with low sperm count. However, these studies found no association between MEP, MMP or MEHP metabolites and low sperm count (Duty et al., 2005; Duty et al., 2003a; Duty et al., 2003b; Hauser et al., 2006).

A study in Shanghai examining concentration of DEHP, DBP and DEP in semen found no associations with sperm concentration but did see a significant correlation between DEHP and impaired sperm structure (Zhang et al., 2006). These contradicting results could be due to the small sample size of the study, or they demonstrate different levels and combinations of exposure for males in Shanghai compared to men from other countries where studies have been conducted, such as Denmark.

Two studies that looked at the entire U.S. population found that sperm DNA damage is associated with exposure to MEP and MEHP (Hauser et al., 2007; Duty et al., 2003b). And a cohort study comparing phthalate metabolites and serum, or blood, hormone levels in people exposed to phthalates at work found inverse relationship between reduced serum free
testosterone levels, increased luteinizing hormone to free testosterone ratio, and concentration of MBP (Pan et al., 2006). While another study comparing phthalate metabolites and hormone levels (follicle-stimulating hormone, luteinizing hormone, sex hormone-binding globulin, testosterone, and inhibin-B) in blood samples found an association between MBzP and decreased follicle-stimulating hormone but no associations between the other metabolites and blood hormone levels (Duty et al., 2005).

The relationship between phthalates, their metabolites, and sperm quality parameters is not fully understood yet, but there is enough evidence to substantiate the claim that exposure phthalates and phthalates mixtures in utero can have negative outcomes on future sperm motility, count, concentration and morphology.

Conclusion

While a substantial amount of research must yet be done to fully understand the effects of phthalates and phthalate mixtures on male reproduction, the evidence that phthalate exposure is associated with negative reproductive outcomes is substantial. Given the substantial evidence that phthalates and their metabolites are linked to androgens, estrogens, and other steroids, further analysis of the relationship between phthalate toxicity and steroidogenesis, or the production of steroids, will prove to be the next challenge in understanding the precise mechanisms by which phthalates cause adverse health outcomes. While it is widely accepted that phthalates inhibit testosterone production, few publications have explored the impacts phthalates have on production of other steroids. Further exploration of phthalate impacts on the steroidogenesis pathway will allow researchers to pinpoint where the phthalates are active and, ultimately, help scientists and policy-makers to make decisions on phthalate regulation.
Another vital next step is fully understanding the relationship between amniotic fluid metabolite levels and maternal urinary metabolite levels. Then NHANES program conducted from 1999 – 2000 found that levels of MEP, MBP, and MEHP in were measured at higher concentrations in maternal urine samples than in amniotic fluid. Though the urine and amniotic samples were not paired in this survey, other studies have supported this trend of higher metabolite levels in maternal urine than in amniotic fluid (Barr et al., 2003; Calafat et al., 2006). It has been suggested that certain fetal enzymes do not full function fully in neonates preventing the fetus from metabolizing the phthalates (NRC, 2008).

Sperm quality and motility studies must also progress in order to understand the degree to which phthalates impact semen parameters. The decline in sperm quality and concentration that has been observed over the past 60 years could have serious implications on human fecundity. This declining trend must be thoroughly analyzed to ensure male sperm quality does not reach a critically low point.

The pervasiveness of phthalates is highly concerning given the well supported evidence of phthalate syndrome along with the substantiated associations between phthalates and cancer (NRC, 2008). Regulations on phthalate use in industrial and commercial applications however, are minimal at present because producers of these phthalate-containing products do not want to remove the effective and relatively inexpensive chemicals from their products. Individual phthalates cannot be regulated or banned without extensive research to support claims of toxicity. Unfortunately, epidemiology studies are costly and time-consuming so the process of regulating a chemical is lengthy. With the help of developing technology, such as the Federal Environmental Protection Agency’s Toxicity Forecaster (ToxCast™) which uses high-throughput screening assays to analyze large numbers of chemicals quickly and efficiently. The
high-throughput screening assays expose live cells or isolated proteins to chemical under review. Researchers then evaluate any biologic activity in the assays which could indicate toxicity. New methods of risk and toxicity assessment such as ToxCast™ will allow scientists to determine adverse health outcomes of specific chemicals much more rapidly than with current epidemiology study protocol. Perhaps, one day, toxicologists will be able to assess the toxicity of new chemicals as quickly as they are created.
Appendix

Figure 1: Fetal androgen insufficiency and common adverse outcomes (NRC, 2008)
**Figure 2:** Phthalate susceptible pathways during development of the male reproductive tract and the associated types of hypospadias (condition in which urethra opening occurs on the underside of the penis instead of at the tip that has been associated with high maternal phthalate levels). The “X”s show the sites of phthalate sensitivity and disruption of reproductive tract formation. Percent prevalence of each type of hypospadias is shown in parenthesis (Chong, 2011)

<table>
<thead>
<tr>
<th>Chemical (CAS-RN)</th>
<th>Acronym</th>
<th>Common Uses</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl phthalate (131-11-3)</td>
<td>DMP</td>
<td>Paint, coatings, rubber, nitrocellulose, solvent for cosmetics and personal care products, household cleaners, encapsulation of electrical wiring, children’s toys, and insect repellent (EPA, 2010; NICNAS, 2008c; Godwin 2010)</td>
<td><img src="image" alt="Structure of DMP" /></td>
</tr>
<tr>
<td><strong>Monomethyl phthalate (4376-18-5)</strong></td>
<td>MMP</td>
<td>Metabolite; environmental degradation product</td>
<td></td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Diethyl phthalate (84-66-2)</strong></td>
<td>DEP</td>
<td>Fixative in cosmetics; detergents and insecticides; plasticizer in medical and consumer products; solvent for cellulose acetate used in food packaging (Contzen, 2006; Fuji, 2005)</td>
<td></td>
</tr>
<tr>
<td><strong>Diallyl phthalate (131-17-9)</strong></td>
<td>DAP</td>
<td>Particle board in furniture and walls; plasticizer in polyesters; molding, electrical parts, laminating compounds; impregnation of metal castings (NTP, 1986; Saillenfait, 2008)</td>
<td></td>
</tr>
<tr>
<td><strong>Diisopropyl phthalate (605-45-8)</strong></td>
<td>DIPP</td>
<td>General Plasticizer</td>
<td></td>
</tr>
</tbody>
</table>

**Medium-chained Phthalates**

<table>
<thead>
<tr>
<th><strong>Dibutyl phthalate (84-74-2)</strong></th>
<th>DBP</th>
<th>Plasticizer in resins and polymers, softener in adhesives, lacquers, perfume solvent and fixative, suspension agent in aerosols, lubricant, antifoamer, skin emollient, cosmetics, explosives, rocket propellant (NINCAS, 2008a; NTP 1995)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monobutyl phthalate (131-70-4)</strong></td>
<td>MBP</td>
<td>Metabolite; environmental degradation product</td>
</tr>
<tr>
<td><strong>Diisobutyl phthalate (84-69-5)</strong></td>
<td>DIBP</td>
<td>Solvents, additives, plasticizers in PVC, nitrocellulose, and cellulose ether, wires, cables, coatings, paints, and flooring material (Saillenfait, 2006)</td>
</tr>
<tr>
<td><strong>Butyl benzyl phthalate (85-68-7)</strong></td>
<td>BBP</td>
<td>Vinyl tiles, traffic cones, food conveyor belts, artificial leather, carpet backing, and vinyl foams (CERHR, 2003a; Saillenfait, 2008)</td>
</tr>
<tr>
<td><strong>Monobenzyl phthalate (2528-16-7)</strong></td>
<td>MBzP</td>
<td>Metabolite; environmental degradation product</td>
</tr>
<tr>
<td><strong>Monobutyl phthalate (131-70-4)</strong></td>
<td>MBP</td>
<td>MBP see above</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Dipentyl phthalate (131-18-0)</td>
<td>DPP</td>
<td>Plasticizer in polyvinyl chloride (PVC)</td>
</tr>
<tr>
<td>Dihexyl phthalate (84-75-3)</td>
<td>DHP</td>
<td>Plastisols in automobile parts, flooring PVC, canvas tarps, notebook covers, traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts (CERHR, 2003b; OEHHA, 2007)</td>
</tr>
<tr>
<td>Dicyclohexyl phthalate (84-61-7)</td>
<td>DCHP</td>
<td>Plasticizer for polymers including nitrocellulose, ethyl cellulose, chlorinated rubber, polyvinyl acetate and polyvinyl chloride, heat sealer for cellulose, adhesive manufacturing, and in screen printing inks (NIHCAS, 2008b)</td>
</tr>
</tbody>
</table>

**Long-chained Phthalates**

| **Di(2-ethylhexyl) phthalate (117-81-7)** | DEHP | Plasticizer in PVC, building and construction materials, car products, footwear, food packaging, children’s products, and medical devices (CERHR, 2000) |
| **Mono(2-ethylhexyl) phthalate (4376-20-9)** | MEHP | Metabolite; environmental degradation product |
| Dioctyl phthalate (117-84-0) | DOP | Plasticizer, flooring materials, carpets, tarps, pool liners; indirect food additive as a component of seam cements, bottle cap liners, and conveyor belts (CERHR, 2003d) |
| Di(isononyl) phthalate branched (68515-48-0) | DINP | PVC plasticizer, automobiles, building materials, consumer products, and toys (CERHR, 2000; Kavlock, 2002) |
| Octyl decyl phthalate (119-07-03) | ODP | Plasticizer for vinyl resins, indirect food additive as a component of adhesives (Chemical Dictionary) |
| Diisodecyl phthalate (68515-49-1) | DIDP | Primarily PVC plasticizer, rubbers, resins, and non-polymer uses including anti-corrosion paints, anti-fouling paints, lacquers, inks, adhesives and sealants (CERHR, 2003c; EU, 2003; Kato, 2007) |
Diisodecyl phthalate (85507-79-5)  DIUP  Plastic packaging materials, unlamented film and sheet, flame-resistant plastic, petrochemicals, resin, and synthetic rubber, automotive sealant/adhesives, and in electrical, electronic, rubber, and plastic products (NINCAS, 2008d)

Size is classified as long, medium, or short based on the number of carbons in the side chain of each chemical. Long: ≥ 7 carbon side chain; medium: 4 - 6 carbon side chain; short: ≤ 3 carbon side chain. Metabolites were not included in chain length groupings or grouping analyses.

**PHTHALATES, GROUPINGS, COMMON USE AND STRUCTURE**

**Table 1**: Common phthalates and their respective abbreviations, CAS-RN number, size class, common uses, and chemical structure.

![AGD Distribution by Gender](image)

**Figure 3**: Distribution of anogenital distance (mm) in boys and girls (Swan et al., 2008)
References


