YOUNG INVESTIGATOR REVIEW

Endocrine-disrupting chemicals and male reproductive health

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Abstract Endocrine-disrupting chemicals are substances present in the environment that can interfere with normal hormonal balance and thus exert potentially adverse health effects on the human organism. Male reproductive system development and function may be susceptible to the effects of such environmental toxicants. Bisphenol A, phthalates and alkylphenols are important components of multiple products and are thus ubiquitously present in the environment. It has been demonstrated under laboratory conditions that they can exert detrimental effects on the male reproductive system. However, human exposure data are scarce and do not uniformly support toxicity of these substances at environmental concentrations. Despite substantial research efforts, the final answer to the problem of endocrine-disrupting chemicals is not yet in sight.© 2013, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: alkylphenols, bisphenol A, endocrine-disrupting chemicals, male reproductive health, phthalates

Introduction

Endocrine-disrupting chemicals are a heterogeneous group of substances that began to attract attention two decades ago due to possible harmful effects (Colborn et al., 1993). According to the US Environmental Protection Agency, an endocrine-disrupting chemical is defined as ‘an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes’ (Kavlock et al., 1996). Considering reproductive function, most of the effects are exerted through disturbance of oestrogen- or androgen-mediated processes. In many reports written in the last two decades, increasing exposure to these substances has even been proposed as the mechanism for decreasing male reproductive function and lower average sperm counts, although the hypotheses about deteriorating male reproductive capabilities are controversial (Andersson et al., 2008; Carlsen et al., 1992; Jouannet et al., 2001; Safe, 2012). The use of some of the established toxic substances, such as polychlorinated biphenyls or
polybrominated diphenyl ethers, has been restricted or even banned in the Western world. Nonetheless, many of these substances can still be detected in considerable concentrations in the environment (Rudel and Perovich, 2009). Moreover, unresolved controversies around certain other compounds, including bisphenol A and phthalates, have resulted in increasing exposure to these chemicals in the last decades.

This brief review will critically investigate the possible effects of bisphenol A, phthalates and alkylphenols on the male reproductive system and current research efforts. First, the production, use and sources of exposure to discussed endocrine-disrupting chemicals will be presented. Second, possible mechanisms of action and demonstrated effects in laboratory conditions will be discussed. Finally, the current evidence of possible effects of bisphenol A, phthalates and alkylphenols on the human male reproductive system is reviewed.

**Production and human exposure**

Bisphenol A, phthalates and alkylphenols are important components of many industrial processes. Although exact quantities are difficult to estimate, it is reckoned that around 6 million tonnes of phthalates are produced worldwide every year (Rudel and Perovich, 2009). For bisphenol A, estimates range 2.2–4.7 million tonnes, of which around 1.2 million tonnes are produced in the EU, and the amounts are rising by about 6–8% yearly (Fernandez, 2010; Huang et al., 2012). The annual production of alkylphenols has been estimated to be 154,000 tonnes in the USA and 75,000 tonnes in the EU (Soares et al., 2008). Since these data were published, the use of alkylphenols has been restricted in the EU, but they are still found in considerable concentrations in the environment (Soares et al., 2008). All of these substances or their metabolites have been detected in human urine, serum, amniotic fluid of pregnant women, in breast milk and even in semen (Calafat et al., 2005; Guenther et al., 2002; Huang et al., 2009; Main et al., 2006).

Bisphenol A is one of the most investigated and, for many authors, one of the most potent endocrine-disrupting chemicals (Maffini et al., 2006). It is extensively used in polycarbonate plastic and epoxy resin production. Thus, bisphenol A can be found in plastic water bottles, food containers, a variety of household products (e.g. compact disks, consumer electronics), medical equipment (e.g. dental fillings) and thermal paper. Human exposure is widespread, as every large biomonitoring study has detected bisphenol A in more than 90% of tested samples (Vandenberg et al., 2010). The largest yet-performed study, the US National Health and Nutrition Examination Survey detected bisphenol A in the urine samples of 92.6% of US males (Calafat et al., 2008). Current knowledge suggests that the oral route is most important for bisphenol A exposure (Geens et al., 2012). However, several authors suggest that alternative routes, such as inhalation or transdermal absorption, may be underestimated sources of exposure (Stahlhut et al., 2009).

Diesters of 1,2-benzene dicarboxylic acid (phthalic acid), commonly referred to as phthalates, are a group of substances widely used in industry and thus ubiquitously present in many everyday products. High-molecular-weight phthalates (e.g. di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate and di(n-octyl) phthalate) are mainly used as plasticizers in the manufacture of flexible vinyl, whilst low-molecular-weight phthalates (e.g. diethyl phthalate and dibutyl phthalate (DBP)) are used in personal care products. Thus, they can be found in food containers, vinyl upholstery, adhesives, perfumes and eye shadow. However, they are not covalently bound to plastic material and consequently can be released into the environment with time and the use of products. Like bisphenol A, their metabolites can be widely detected in the population. Thus metabolites of the parent compounds have been found in the vast majority of males in the USA (Silva et al., 2004). Similarly to bisphenol A, the exposure to phthalates is primarily through oral intake, although transdermal route or inhalation may contribute significantly (Rudel and Perovich, 2009).

Alkylphenol ethoxylates are a group of substances most commonly used as surfactants in common consumer products, such as detergents, disinfectants, surface cleaners, cosmetic products, spermicides and pesticides. The most important members of this group are nonylphenol ethoxylate and octylphenol ethoxylate (Tubau et al., 2010). These substances undergo metabolic breakdown in the environment and lose ethylene oxide side chains to become alkylphenols (4-n-octylphenol and 4-n-nonylphenol). Unlike most of the exogenous chemicals, which usually become less toxic with biodegradation, alkylphenols actually increase their toxicity during this process. Alkylphenols are frequently found in wastewaters (White et al., 1994; Ying et al., 2002) and are also present in food (Guenther et al., 2002). Ingestion routes have not been definitely described, but primarily oral ingestion and secondarily inhalation and transdermal route are most likely (Wilson et al., 2001).

**Mechanisms of action on the male reproductive system**

A disturbance of the male reproductive system can take place at different periods of a lifetime. In order to study disturbances, it is important to consider first what is known of the physiological mechanisms that ultimately lead to healthy sperm production. The development of the male reproductive system requires the activation of specific pathways by hormones, notably androgens and anti-Müllerian hormone. Thus, although testis formation itself is not hormone dependent, most other aspects of masculinization depend on normal testicular hormone production. Furthermore, testis cell development (as opposed to testis formation) is dependent on the local action of hormones (Sharpe, 2001). Androgens are the most important hormones in the normal development of Wolffian ducts that differentiate into epididymis, vas deferens and seminal vesicles (Wilson, 1978). Dihydrotestosterone, which is produced locally from testosterone by 5α-reductase, is the most important hormone in masculinization of external genitalia and prostate (Fisher, 2004). Hence, a balanced hormonal environment is essential for a normal development of the male genitourinary tract. Hormonal disturbances have been linked to masculinization anomalies, but little research has been performed to relate early hormone
imbalances to long-term testicular function in terms of fertility (Sharpe, 2001). It seems likely that the abnormal development of testes in fetal and neonatal life can have long-term consequences for sperm production (Sharpe, 2001). It has also been surmised that prepubertal exposure to endocrine-disrupting chemicals is more likely to have negative effects on reproductive function, because the blood–testis barrier in humans is developed just before puberty (Latini et al., 2006).

With these points in mind, the effects of endocrine-disrupting chemicals mediated through the activation or the inhibition of androgen and oestrogen receptors are of primary concern for male reproductive function. However, action through the aryl hydrocarbon receptor, which is a cytosolic transcription factor with roles in developmental processes, xenobiotic metabolism and immunological responsiveness may also be of importance (Bonefeld-Jørgensen et al., 2007). Besides the direct action through nuclear or membrane receptors, oestrogenic endocrine-disrupting chemicals can also exert more general effects through the induction of oxidative stress (Anderson et al., 2003; Aydoğân et al., 2008; Chitra et al., 2003; Gong and Han, 2006).

Furthermore, possible negative actions on pregnancy via epigenetic toxic mechanisms have recently been suggested. Epigenetic modifications involve heritable changes in gene expression without changes in DNA sequence. These changes include DNA methylation alterations, histone modifications and the expression of non-coding RNA. The early developmental period is the most susceptible to epigenetic mechanisms because the rate of DNA synthesis is the highest. Studies have been performed that demonstrate possible epigenetic actions of bisphenol A and phthalates in rodents and even in human cell cultures (Singh and Li, 2012), observations that further expand the possible toxic mechanisms of endocrine-disrupting chemicals, but which are yet to be verified in human studies.

Animal studies

Bisphenol A

Bisphenol A has been considered as a prototypical non-steroidal oestrogen that interferes with nuclear oestrogen receptors in several targets in the body. The affinity of binding to the oestrogen α and β receptors however, has been demonstrated to be weak, in some studies even up to 1000–100,000 times lower than 17-β-oestradiol (Welshons et al., 2003). bisphenol A is reported to cause its effects by interfering with both androgen production and function (Akingbemi et al., 2004; Lee et al., 2003; Paris et al., 2002; Roy et al., 2004). It has also been shown to have the ability to impair Sertoli cell function by interfering with expression and localization of tight junction proteins (Fiorini et al., 2004; Li et al., 2009; Salian et al., 2009). Furthermore, not only direct, but also indirect actions through the induction of epigenetic mechanisms, with most studies showing evidence of DNA hypomethylation, have been described (Doshi et al., 2011; Singh and Li, 2012).

In laboratory rodents, prenatal, perinatal and adult exposure to bisphenol A by the oral route or subcutaneous injections has been shown to cause developmental genitourinary anomalies, decreased epididymal weight, daily sperm production or increased prostate weight even when exposed to doses lower than 50 mg/kg/day, which is currently accepted as the lowest observed effect dose used to calculate the acceptable daily intake in humans (Nagel et al., 1997; Richter et al., 2007; Salian et al., 2009; vom Saal et al., 1998; Williams et al., 2001). When considering doses significantly lower than 50 mg/kg/day, exposure of pubertal rats and mice to 3 mg/kg/day of bisphenol A by subcutaneous injections, resulted in lower concentrations of epididymal spermatozoa and testosterone (Herath et al., 2004) whilst in adult mice, lower concentrations of epididymal spermatozoa were observed even when exposed to as low as 25 μg/kg/day of bisphenol A by oral route (Al-Hiyasat et al., 2002). When injected subcutaneously at 1 mg/day, bisphenol A resulted in lower testosterone concentrations along with higher LH concentrations in adult rats (Tohei et al., 2001). Contrary to these findings, several studies demonstrated no negative reproductive effects at doses as low as 0.2 μg/kg/day or even as high as 5 mg/kg/day administered by the oral route (Ema et al., 2001; Tinwell et al., 2002; Tyl et al., 2002). A recent study by Howdeshell et al. (2008a) reported that no negative reproductive effects on male offspring could be observed when pregnant rats were gavaged with 2–200 μg/kg/day of bisphenol A in contrast to ethinyl oestradiol exposure. The same group subsequently reported that gestational exposure to bisphenol A did not result in negative developmental effects on female rat offspring (Ryan et al., 2010). Because these results directly oppose the bisphenol A oestrogenic effects demonstrated in early studies, it has been suggested that concerns about possible bisphenol A oestrogenicity at environmental concentrations should end (Sharpe, 2010). Hence, it can be concluded that evidence suggesting low-dose bisphenol A exposure has an effect on the male reproductive system in laboratory conditions is unconvincing.

Phthalates

Phthalates are considered to be one of the major groups of anti-androgenic substances and thus are established reproductive and developmental toxicants (Grady and Sathyanarayana, 2012). They can exert their anti-androgenic action by directly inhibiting testosterone synthesis in Leydig cells, which has been proposed to be a result of cytochrome CYP 17 dysfunction (Foster, 2005). Some phthalates have also been shown to disrupt the patterns of gene expression that regulate cholesterol and lipid homeostasis or insulin signalling, which could also result in lower testosterone synthesis (Barlow et al., 2003; Liu et al., 2005). Even a weak action via oestrogen receptors has been demonstrated for some phthalates (Harris et al., 1997).

It has been shown that rats exposed to phthalates during the prenatal period can develop specific developmental reproductive anomalies that have been identified as ‘phthalate syndrome’. These include cryptorchidism, smaller testes and penis size and alterations to the vas deferens and epididymis as well as a shorter anogenital distance (Foster, 2006). A dose-dependent correlation between DBP administered during pregnancy and lactation, reduced anogenital distance (Mylchreest et al., 1998) and
lower testosterone concentration has been reported at doses as low as 50 mg/kg/day (Lehmann et al., 2004). DEHP exposure in juvenile rats has been correlated with increased testicular apoptosis and the loss of seminiferous epithelium when administered at 2 g/kg/day (Park et al., 2002). Moreover, prepubertal rats have been shown to be more susceptible to DEHP-induced changes than adult rats. Prepubertal rats treated with as low as 10 mg/kg/day DEHP for 14 days have produced lower concentrations of testosterone, whilst adult rats have not shown any differences (Akingbemi et al., 2001). However, paradoxically, with extended, 28-day exposure, an increase in serum testosterone concentration associated with Leydig cell hyperplasia was observed in prepubertal rats (Akingbemi et al., 2001, 2004).

Alkylphenols

Nonylphenol and octylphenol are substances with weak oestrogen-receptor binding potency (1000–1,000,000 lower than 17-β-oestradiol) (Preuss et al., 2010; White et al., 1994). They can be present in the environment in a range of isomers. It has been shown for nonylphenol that isomers vary in their oestrogenic potency (Gabriel et al., 2008; Preuss et al., 2006). However, the identification and quantification of each isomer presents an additional difficulty when investigating their effects (Ying et al., 2012). Additionally, antagonistic effects upon androgenic receptors have been demonstrated (Lee et al., 2003; Paris et al., 2002; Roy et al., 2004).

The effects of endocrine disruption by alkylphenols have been studied extensively in laboratory rodents. An early, multigenerational study by Chapin et al. (1999), examined the male and female offspring of pregnant rats treated for 4 weeks with high (200–2000 parts per million, corresponding to 9–350 mg/kg/day) doses of 4-n-nonylphenol for up to the third generation. It was concluded that 4-n-nonylphenol has only minor effects on reproductive system parameters in the offspring of tested rats at the highest doses applied. A later study confirmed the observation of a possibly lower epididymal weight in the male offspring at higher 4-n-nonylphenol dose exposure (15–75 mg 4-n-nonylphenol/kg/day for 8 days) (Hossaini et al., 2001). Neonatal exposure (8 mg/kg/day for up to 15 days post partum) of rats by intraperitoneal application of 4-n-nonylphenol did not result in developmental reproductive anomalies (Odum and Ashby, 2000), but exposure of juvenile rats to administration of 100 mg 4-n-nonylphenol/kg/day for 30 days resulted in significant testicular damage and a reduction in spermatogenesis (Tan et al., 2003). It has also been shown that 4-n-octylphenol can induce a lower epididymal sperm count in pubertal rats at the low-dose exposure of 3 mg/kg/day for 2 weeks (Herath et al., 2004). A significantly increased rate of Sertoli cell apoptosis was observed when cultured in vitro with 4-n-nonylphenol for 72 h at the high concentrations found in the environment (Wang et al., 2003). Also, rats treated with high doses of 4-n-nonylphenol by gavage (250 mg/kg/day for 50 days) subsequently exhibited both lower epididymal weight and sperm count compared with controls (Han et al., 2004). Various 4-n-nonylphenol isomers have also been shown to be able to inhibit testosterone biosynthesis in rats (Laurenzana et al., 2002; Ying et al., 2012). However, a few in-vitro studies suggest a biphasic effect with an increase of testosterone production at low-dose 4-n-octylphenol or 4-n-nonylphenol exposure and a decrease of testosterone production at high-dose exposure (Murono et al., 1999; Wu et al., 2010).

The data lead to the conclusion that exposure to high concentrations of alkylphenols probably produces various negative effects on the male reproductive system in laboratory conditions. As well as for other endocrine-disrupting chemicals, including bisphenol A and phthalates, low-dose exposure presents with inconclusive results. Considering that exposure to endocrine-disrupting chemical in the environment occurs at low doses, this presents a special challenge in the design of human population studies.

Human studies

Bisphenol A

Reviewing the studies on human populations, the first data on bisphenol A were published in 2002 by Takeuchi and Tsutsumi (2002), who interestingly demonstrated a positive correlation between bisphenol A exposure and total/free testosterone values in men and women. It has also been shown that occupationally exposed men have higher urinary bisphenol A concentrations than controls and that bisphenol A is related to lower FSH concentrations (Hanaoka et al., 2002). A recent study performed on a population of 360 presumptively fertile men (partners of pregnant women) concluded that bisphenol A is inversely correlated with free androgen index concentrations, but no correlation with semen quality was noted (Mendiola et al., 2010). In a subset of 167 men attending an infertility clinic, Meeker et al. (2010a) demonstrated a negative correlation between urinary bisphenol A concentration and inhibin B concentration and oestradiol/testosterone index (a marker of aromatase activity) as well as a positive correlation between urinary bisphenol A and both FSH and FSH/inhibin B ratio (a marker of Sertoli cell function). An additional study by the same group reported a non-significant trend with increasing bisphenol A concentration related to lower sperm concentration, motility and morphology and higher levels of DNA damage (Meeker et al., 2010b). A small study that included 27 couples undergoing IVF even suggested that male bisphenol A exposure might negatively influence embryo development after fertilization of oocytes (Bloom et al., 2011). However, currently available data do not provide enough sound evidence to reveal detrimental environmental bisphenol A exposure effects on male reproductive capability in terms of sperm quality.

Phthalates

In humans, evidence demonstrating a negative action of phthalates on the reproductive tract is also accumulating. A relationship between anogenital distance and maternal urinary concentrations of phthalate metabolites was noted in 85 boys studied by Swan et al. (2005). This study investigated the effect of prenatal environmental exposure to phthalates on genital development in newborns. Mothers classified in the highest quartile of exposure gave birth to sons with shorter anogenital distances compared with
mothers in the lowest quartile (Swan et al., 2005). However, this result was not confirmed in a subsequent smaller study \((n = 33)\) by Huang et al. (2009). A study by Main et al. (2006) found inverse relationships between several phthalate monoesters in breast milk samples and the serum ratio of LH to free testosterone of breastfed boys \((n = 130)\), indicating a possible adverse effect on Leydig cells or the pituitary–gonadal axis; however, no correlation with the incidence of cryptorchidism was observed (Main et al., 2006).

A few studies have shown that exposure to phthalates in adulthood could result in decreased male fertility. Short-term (12-hour) in-vitro incubation of spermatozoa with the highest concentrations of phthalates detected in human semen samples through environmental exposure (13.47 \(\mu\)g/ml DBP and 5.73 \(\mu\)g/ml DEHP) resulted in decreased sperm motility, whilst prolonged incubation (96 h) resulted in sperm cytotoxicity (Pant et al., 2011). Recently performed human fetal testis explant studies provide an interesting insight into the potential phthalate disturbance of human male steroidogenesis. It has been demonstrated that DEHP and monoethylhexyl phthalate (MEHP, a metabolite of DEHP), when cultured in vitro with adult human testes explants, can inhibit testosterone production (Desdoits-Lethimonier et al., 2012). Conversely, these observations could not be confirmed in in-vivo studies of human developing testes. Two studies investigating in-vivo DBP exposure to human fetal testes explants xenografted to rodent hosts have recently been performed. Although DBP could suppress steroidogenesis in testes of mice and rats, no effects could be demonstrated in human xenografts for a range of applied DBP concentrations (Heger et al., 2012; Mitchell et al., 2012). However, a negative effect on testicular germ cell development was noted and should be further investigated (Heger et al., 2012).

Only a few epidemiological studies directly investigated the detection of phthalate metabolites and any correlation with human testicular function. One of the first studies investigated 168 men from subfertile couples and concluded that specific phthalate metabolites, when categorized in tertiles, are correlated to lower sperm concentration and that specific phthalate metabolites, when categorized in tertiles, are correlated to lower sperm concentration and motility (Duty et al., 2003). Further studies on this subject have confirmed weak associations between exposure to phthalate metabolites and lower sperm concentration, motility and morphology in adults (Duty et al., 2004; Hauser, 2008; Hauser et al., 2006; Liu et al., 2012; Wirth et al., 2008), although, some have not proved this connection (Herr et al., 2009; Jönsson et al., 2005). It has also been shown that sperm DNA damage may be increased by phthalate exposure (Hauser et al., 2007). Regarding hormonal balance, a study by Meeker et al. (2009) confirmed an inverse correlation between an interquartile increase in MEHP and the testosterone, oestradiol and free androgen indices. Combined with the results from rodent studies, it seems reasonable to conclude that phthalates, with their anti-androgenic effects, could be related to genitourinary developmental anomalies, but more evidence from the human population is needed to validate these results. In the adult, however, a strong relationship between environmental exposure to phthalates and male reproductive function is not evident due to conflicting available evidence.

### Alkylphenols

In spite of a substantial amount of available data on the action of alkylphenols in rodent and marine studies, human evidence is lacking. Studies investigating infant exposure through mothers’ breast milk have shown that exposure to alkylphenols is considerable and can be at the level of tolerable daily intake calculated on the basis of rodent studies (Ademollo et al., 2008). When performing the literature search, however, human epidemiological studies linking alkylphenol exposure to possible health effects were not found.

### Interactions of endocrine-disrupting chemicals

The human environment does not expose us to specific endocrine disrupters, but to a complex cocktail. This fact poses the question: does this cocktail of substances have different effects than exposure to single isolated substances? There are still relatively few studies investigating this scenario. Generally, oestrogenic substances are thought to act in concentration-additive mode, which means that mixture effects can be predicted by each substance concentration and the relative binding affinity to the oestrogenic receptor (Howdeshell et al., 2008b; Sun et al., 2009). However, the recent demonstration that some endocrine-disrupting chemicals at certain concentrations can also act in ‘competitive antagonism on oestrogen receptors challenges this view (Li et al., 2012). Moreover, the complexity of the mechanisms involved in endocrine-disrupting chemical actions means that their health effects can be the result of the action through multiple pathways, potentially leading to greater-than-additive effects (Kortenkamp, 2007). Such interactions could even allow substances that would not produce any effects by themselves to produce significant effects at concentrations present environmentally (Christiansen et al., 2012; Kortenkamp, 2007).

### Conclusions

Exposure to endocrine-disrupting chemicals in the environment is complex. Although the restrictions on the use of evidently toxic substances have been implemented in certain countries, some endocrine-disrupting chemicals and their derivatives can still be widely detected in the environment. After almost 20 years of research, the question that still occupies the scientific community is: how seriously and at what concentrations do endocrine-disrupting chemicals compromise human health? The period of sexual development appears to be most susceptible to the negative effects of environmental pollutants. At least for exposure to phthalates, a higher incidence of developmental anomalies in human populations has been observed. This finding led subsequently to the restriction of their use for children’s accessories in several countries. Similar limitations have been adopted for bisphenol A, although its toxicity potential at environmental concentrations is not definitely proven. Several substances that show negative effects in in-vitro or animal studies have not been proven to exert detrimental effects in humans. Often, they are present in the environment at concentrations several magnitudes lower than the...
exposure under laboratory conditions. However, substances that may not be toxic at environmental concentrations may act synergistically with other endocrine-disrupting chemicals to potentiate their toxic potential. The final answer to whether the toxicity of bisphenol A, phthalates and alkylphenols is of serious concern is not yet in sight and remains to be answered with further studies.

Acknowledgments

The author thanks Veljko Vlaisavljević for all the guidance and support received in his work. The author would also like to thank Martin Johnson for critical readings of the manuscript.

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Declaration: The author reports no financial or commercial conflicts of interest.

Received 15 December 2012; refereed 4 February 2013; accepted 5 February 2013.