Chapter 7

Endocrine Disruptors and Reproductive Health in Males

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Additional information is available at the end of the chapter

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Abstract

Nowadays, endocrine-disrupting chemicals are considered to be one of the main causes of the ever-increasing occurrence of problems with male fertility. These compounds of natural or anthropogenic origin are omnipresent in the environment and organisms are exposed to them practically nonstop through the air, water, food, and occupationally. Endocrine disruptors have the ability to mimic effects of reproductive hormones and demonstrably can interfere with the endocrine system leading to reproductive disorders at different levels, and considering male reproductive functions, most of the impacts are performed by the breakdown of estrogen- or androgen-mediated processes. A significant body of evidence based upon laboratory or wildlife animal experiments and meta-analysis of semen studies in men indicates that exposure to endocrine disrupting compounds is associated with male reproductive malfunctions, including impairment of spermatogenesis followed by reduced semen quality parameters (sperm concentration, motility, and morphology). Alkylphenols, bisphenol, and phthalates are substantial components of many products with which people come into contact daily. This brief review will emphasize on the possible effects of alkylphenols, bisphenol, and phthalates on the male reproductive system, and current research efforts related to these substances mainly in the context of two main processes taking place in testicular tissues—steroidogenesis and spermatogenesis.

Keywords: male, reproduction, steroidogenesis, spermatogenesis, alkylphenols, bisphenols, phthalates

1. Introduction

Over the last years, many epidemiological studies have been observing worrisome trends in the incidence of human infertility rates. Increasing prevalence of congenital

abnorm-alities such as hypospadias and cryptorchidism has also been confirmed by numerous reports. Male fertility generally relies on the quantity and quality of spermatozoa, sufficient activity of Leydig cells, and a proper hormonal balance. Infertility is a widespread problem defined as the inability to conceive after one year of unprotected intercourse. In many cases, there are no obvious signs of infertility. Substantial part of the problem is the disruption of essential cellular processes responsible for normal reproductive functions [1, 2]. Given the short time, genetic changes cannot explain such alterations. We may assume that they only reflect on persistently adverse changes in the environment or in lifestyle. However, it cannot be ignored that some individuals may be more susceptible or resistant to these adverse effects than others, indicating that genetic factors do play key roles [3]. Enormous production and release of industrial chemicals into the environment has led the scientific community to hypothesize that current pollutants may irrefutably disrupt health conditions, leading to extensive damages to physiological functions. In fact, a huge number of chemicals have been found to interact with the endocrine system of different animals in laboratory studies and there is an increasing number of reports on the endocrine disruption in wildlife [4]. Endocrine disruptors (EDs) are an extremely heterogeneous group of ubiquitous synthetic substances, environmental pollutants, and commercial products. They are able to alter functions of the endocrine system, inhibit critical cellular processes, increase the risk of hormone-dependent malignancies, and may result in a wide array of adverse health effects. The term endocrine disruption has been adopted by the vernacular of scientists, toxicologists, and appears here to stay [5, 6]. There are many varied sources of EDs. Typical human exposure occurs with respect to the environmental contamination of the food chain, contact with contaminated household dust, and during the use of personal care products. Other EDs are used as industrial lubricants, solvents and high amounts of EDs were found in household products, pesticides, herbicides, detergents, beverage and food storage containers, metal cans, epoxy resins, etc. Many textiles contain contaminants, such as flame-retardants, including tetrabromobisphenol A and polybrominated diphenyl ethers [7]. Although a chronic exposure to ED takes place through the skin contact or inhalation, the major source are food products. Some experimental studies assume that plastic packaging is the largest source of EDs in the human diet. Repeated exposure of food – contact materials to UV light, acid or alkaline contents and heat may cause polymers to breakdown into monomers as phtha lates, which then leach into the food or beverages [8]. Other by-products such as alkylphenols, bisphenols, polychlorinated biphenyls, dioxins or phthalates are ubiquitous and there is a growing concern that living in an ED-contaminated environment may initiate adverse health effects. Detection of ED residues in human serum, seminal plasma, and follicular fluid has raised concerns that environmental exposure to EDs may be affecting human fertility [9]. Nowadays, some of EDs have been banned or otherwise removed from the industrial processes years ago. On the other hand, these are persistent in the environment throughout many years. A wide range of industrial PCB compounds may be still found in pronounce quantities in the environment, although their manufacture was banned in 1977 [10]. Indeed, humans and wildlife are continually exposed to copious potentially hazardous substances that are released into the environment at an alarming rate.

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2. Male reproductive system as a major target of EDs

In this context, possible adverse effects of EDs have been taken into focus, both regarding the effects of EDs on the male reproductive system and with respect to its differential susceptibility towards these compounds. Although there has been an effort to list and rank all possible EDs, the number of evaluated chemicals remains limited. Such information and associated concerns regarding the ubiquitous presences of EDs in the environment have sparked discussions regarding the need for strategies to assess and regulate chemicals with endocrine disrupting properties in order to protect human and wildlife health. During the last years, some epidemiological studies have been comparing an increase in the incidence of male reproductive disorders in many countries. The results showed that the global average sperm count dropped by half and that the sperm motility/viability significantly decreased. In addition, many types of reproductive tract abnormalities were observed in several countries [12]. Several experimental studies have found associations between poor semen quality and increased levels of EDs in the environment [13, 14]. EDs may disrupt not only spermatogenesis, by interfering with germ cells and sperm-supporting cells, but may also affect steroidogenesis occurring in Leydig cells. Many researchers have focused on the potential sources of EDs and their pathological consequences on reproductive health as well as ethnologies in the environment.

2.1. Alkylphenols and their impact on steroidogenesis and spermatogenesis

As we mentioned before, environmental exposure to EDs may adversely affect human and wildlife reproductive functions. Many environmental contaminants including alkylphenols are widely used in the preparation of agrochemicals, industrial and household detergents, paints, and plastics [15]. Alkylphenol ethoxylates, a class of nonionic surfactants, are microbially degraded into alkylphenol diethoxylates and alkylphenol monoethoxylates. These are subsequently degraded into alkylphenols (4-octylphenol; 4-nonylphenol) and along with other subproducts, are known to persist in the environment for a long time [16]. Alkylphenols are endocrine-disrupting agents with native estrogen-like structure and show estrogenic activity. This activity is mediated through the binding of these environmental estrogens to estrogen receptors. Previous studies suggested that estrogenic activity of alkylphenols is linked to a tertiary branched α-carbon and the length of the side chain at that position. Therefore, many experimental studies have investigated estrogen receptor binding and subsequent pathological changes in male reproductive functions. The mechanism also involves interaction with steroidogenic enzymes, transport proteins, and cell signaling processes. However, little is known about the direct effect of alkylphenols on the steroidogenic enzymes (3β-HSD and 17β-HSD) and gene expression [17].

2.1.1. Nonylphenol

One of the most commonly used alkylphenol is nonylphenol (NP). Due to its wide usage, a large amount of nonylphenol is widespread in the environment, especially into water sources. Vazquez-Duhalt et al. [18] have been convinced that the concentration of 0.1 μg/L evokes a

public health risk. Based on this knowledge, several studies have investigated the potential impact of NP on male reproductive functions.

Ying et al. [19] demonstrated that nonylphenol's isomers had different effects on the release of steroid hormones in rat Leydig cells. However, all experimental doses had an unfavorable impact. Specifically, the inhibitory effect of p363-NP isomer was found to be as much as 1.26 times higher than the others. The results imply that the effects of different nonylphenol isomers on the testosterone production do not appear to be completely mediated through the estrogen receptor α or β. For the steroidogenesis, ensured by Leydig cells is an essential conversion of cholesterol into various steroid classes, where 3β-HSD, 17β-HSD, and StAR are responsible for the rate-limiting step. PCR analysis showed that the decrease of testosterone production may be explained by the drastic inhibition of StAR and 3β-HSD gene expression. In a recent study, Wu et al. [20] demonstrated that NP increased testosterone production in rat Leydig cells. The concentration of 127.5 μM NP stimulated the steroidogenic process by elevating the activity of $P450_{scc}$ and stimulating protein expression of StAR. During the same experiment, trypan blue assay was performed. The authors observed the cytotoxic effect of the highest doses of NP (425 μM). Lower experimental doses (42.5–127.5 μM) used in this study had no cytotoxicity until 4 h cultivation. In a previous study, Jambor et al. [21] evaluated the potential impact of NP on the biosynthesis of steroid hormones, cell viability, and ROS production. The production of steroids, specifically dehydroepiandrosterone, androstenedione, and testosterone was reduced following exposure to NP after 44 h of *in vitro* cultivation. Furthermore, the treatment to NP caused a significant intracellular accumulation of ROS in mice Leydig cells. Majdic et al. [22] reported that NP has an inhibitory effect on P450c17, which is essential in the testosterone synthesis. Several studies demonstrated that NP treatment increased apoptosis of testicular cells, including germ and Sertoli cells [23, 24]. According to Han et al. [25], the highest experimental concentration of NP (250 mg/kg/day) may significantly increase the number of apoptotic cells following *in vivo* exposure of male rats. Recent evidence also confirms that NP exposure rapidly increases the apoptosis of Sertoli cells in a dose-dependent manner *in vitro.* The results of flow cytometric analysis indicate that the proportion of apoptotic cells was significantly increased at 20 and 30 μM of NP [26]. *Gap junctional intercellular communications (GJIC)* were shown to be present between adjacent TM4 Sertoli cells [27]. An important role of *GJIC* is to regulate cell growth and differentiation and it is also critical for coordinating steroidogenesis and spermatogenesis. *Gap junctions* are pores composed of connexins (Cx). Several reports indicate that Cx43 is essential for normal testicular functions [28]. Aravindakshan and Cyr [29] showed that the exposure to NP dramatically inhibited *GJIC.* A significant reduction was observed at 10 μM of NP (almost 80%). The effect of NP on the Cx43 expression was dose- and time-dependent. Time-response analyses in which cells were exposed to 10 μM NP indicated that there was a decrease in Cx43 after 24 h. Exposure of TM4 cell line to NP resulted not only in a decrease in the CX43 levels but also a progressive effect on the level of renewal of the connexins, or on their synthesis, or both was confirmed. In addition, epidemiological studies have reported numerous other adverse effects of nonylphenol on the reproductive system, including reduced testis weights, spermatozoa abnormalities, and a decreased sperm production [30, 31].

NP is considered to be an endocrine disrupting compound which could be involved in declines of both quantity and quality of spermatozoa in adult men [32, 33]. A lot of experiments show an

*in vitr*o NP inhibition of sperm motility and viability [34, 35], while *in vivo* studies confirm spermatotoxicity, spermatogenesis failure, reduced sperm counts and motility, seminiferous tubule degeneration including decreased diameters of seminiferous tubules, lumen and epithelial thickness leading to testicular atrophy [36], and abnormalities in sperm morphology following NP exposure [37, 38]. Huang et al. [39] observed detrimental activity of NP on prepubertal Sprague–Dawley male rats under *in vivo* and also under *in vitro* condition, when the animals were treated with 25–100 mg/kg/day for 30 consecutive days by an intraperitoneal injection of NP. NP exposure induced the sperm toxicity, resulting in cell damage and reproductive disorders and initiated oxidative stress, disturbed the PI3K/AKT/mTOR pathway, induced apoptosis and autophagy, and caused developing reproductive damage *in vivo* and *in vitro.* Uguz et al. [40] designed an *in vitro* study with epididymal rat sperm, observed NP-induced (250–500 μg/mL; 1–4 h exposure) impairment of sperm motility, and a decreased mitochondrial membrane potential which probably plays a key role in the malfunction of spermatozoa. Another *in vitro* experiment with ram and boar spermatozoa provides similar results, when exposure of both sperm types to 250 and 500 μg/mL was harmful to progressive motility, percentages of ram and boar sperm with high mitochondrial membrane potential decreased significantly following exposure to concentrations ≥250 μg/mL. Unlike chromatin integrity, which did not seem to be changed after NP administration, there was a dose-dependent activity of NP on the acrosomal integrity of both species at as low as 1 μg/mL for boar sperm and 10 μg/mL for ram sperm 35]. Lukac et al. [41] used a cell model of bovine spermatozoa to determine the effect of NP (1, 10, 100, and 200 μg/mL) on the motility and viability of spermatozoa during several time periods. The results showed a decreased spermatozoa motility and viability in all experimental samples following the addition of NP after 6 h of exposure. The effects of NP were also evaluated in frozen-thawed bull spermatozoa, when the cells were exposed to concentrations of NP at doses 1, 10, 100, 250, and 500 μg/mL. Sperm parameters were assessed at cultivation times of 0, 1, 2, 3 and 4 h and both motility and mitochondrial membrane potential of sperm cells decreased at concentrations ≥250 μg/mL. In addition, the acrosome reaction was induced even at the lowest concentration of NP [42]. Ergun et al. [43] showed that 100 μg/mL NP induced apoptosis by causing DNA breaks in bovine spermatozoa. Vitellogenesis is a sensitive biomarker of xenoestrogen exposure *in vitro* and *in vivo* and vitellogenin is considered to be a key in indicating the presence of xenoestrogens in the environment, as these chemicals have been found to induce the production of this yolk protein in males leading to the impairment of male sexual organ development and disruption of male fertility [44]. NP is estrogenic also to aquatic organisms and experiments related to fish and amphibians have shown that NP is able to induce vitellogenin in the gonads, violating the development of the embryo and larvae, and results in a strikingly skewed sex ratio in aquatic organism via modulating the effects of sex hormones [45]. NP has been connected with the development of different types of sexual dysgenesis in the laboratory and wild fish [46, 47]. Feng et al. [45] investigated the *in vivo* and *in vitro* effects of NP on the motility parameters and fertilizing ability of *Bufo raddei* during amplexus and fertilization period. Based on the results, ROS induced via NP and NP itself was associated with the decrease of the fertilization rate, when *in vitro* assays showed a direct exposure of sperm to NP with a significant impairment of motility, integrity, and increased ROS levels. Negative correlations were observed between motility of spermatozoa and corresponding ROS concentrations, but the level of NP that admittedly affected spermatozoa in this study (200 μg/L) was about 2.5 times of the highest NP level found in natural aquatic environments (0.065–83 μg/L).

2.1.2. Octylphenol

Numerous reproductive issues such as an increased incidence of testicular cancer, lower spermatozoa activity, and disruption of the steroidogenic process have been related to exposure to alkylphenols. One of the greatly widespread alkylphenols is octylphenol (OP). It is used as a component of emulsifiers, detergents, paints and many other synthetic products. Nowadays, OP is mainly present in sediments, surface waters, and even drinking water. Due to its relative stability and hydrophobic properties, OP is bioaccumulated in various tissues and poses a large health risk for the organism [48–50]. It has been reported that certain doses of OP may negatively affect cellular processes such as steroidogenesis and spermatogenesis essential for a normal development and functions of the male sex. However, there are still limited information about the mechanism, through which OP affects biosynthesis of steroid hormones. Some experimental studies have hypothesized that OP may directly modulate the activity of steroidogenic enzymes. Murono et al. [51] documented that exposure to 2000 nM OP affected the testosterone production in rat Leydig cells. In response to the experimental dose, testosterone levels significantly increased after 2, 4, and 8 h cultivation, when compared with the control. Exposure to shorter periods (0.5 and 1 h) were also examined; however, the weak increase at these times was not statistically significant. The increase in hormone production was not associated with changes in cAMP levels and it did not involve the estrogenic activity (binding) to the estrogen receptors. Furthermore, higher testosterone secretion was not the consequence of inhibiting 5α-reductase activity in Leydig cells. Although these results did not describe signaling pathways, it is necessary to identify the potential mechanisms through which intermediate stages of steroidogenesis may be affected. Some epidemiological studies imply that the inhibiting effects of OP on the steroidogenesis are mediated through the potential of OP to generate ROS and inhibit testosterone secretion. Cytochrome $P450_{scc}$ and P450c17 are essential in converting cholesterol to testosterone in Leydig cells. During the steroidogenic process, ROS are produced by electron leakage outside the electron transfer chains and these radicals may cause lipid peroxidation to inactivate P450 enzymes [52]. Several reports evaluated the potential effects of OP on the steroid hormone synthesis [51, 53]. According to Kotula-Balak et al. [54], independently of the incubation time, high doses of OP significantly inhibited the progesterone production in mice MA-10 cells. Inhibition in progesterone levels was significantly higher in the experimental groups cultivated with OP for 3 h than in cells incubated for 12 h. This can be related to the restoration of Leydig cell steroidogenic function within the time of culture. Decreased progesterone production could be mediated through the inhibition of 3β-HSD since it was reported that estradiol inhibits the progesterone level via the disruption of the 3β-HSD function. Murono et al. [55] investigated the impact of OP on the biosynthesis of steroid hormones in rat Leydig cells *in vitro.* The authors reported a biphasic effect, where the lower experimental doses (1 and 10 nM) increased the testosterone production by approximately 10–70% above the control group, whereas higher concentrations (100 and 200 nM) decreased the testosterone level progressively. The inhibitory effect of OP was also evaluated by Nikula et al. [53]. Inhibition of testosterone secretion by 4-*t*-octylphenol in cultured mice Leydig cells has been suggested to occur at the 17β-HSD step. It has also been reported that the gestational exposure of pregnant rats to OP decreases the amount and activity of the P450c17 steroidogenic enzyme in male

offspring and SF-1 (steroidogenic factor) involved in the gonad development and expression of steroidogenic enzymes [56]. Based on the evidence gathered from the literature, it seems possible that inhibited functions of a male reproductive system might be mediated not only through the disruption of steroidogenic enzymes but also via the direct toxic effect of OP, resulting in a lower cell viability and apoptosis. Qian et al. [50] evaluated the cytotoxic effect of OP (30–60 μM) in rat Sertoli cells after a 24 h exposure. Cell viability was significantly reduced at 40, 50, and 60 μM OP. Additionally, the highest experimental dose decreased the Sertoli cell viability in a time-dependent manner with a significant decrease following a 12 h cultivation. The cytotoxic effect of OP is strongly dependent on the experimental doses. Jambor et al. [57] evaluated the *in vitro* effect of 4-OP on mice Leydig cell viability. The results showed a greater viability at 1, 2.5 and 5 μg/mL of 4-OP following 44 h of cultivation. Kotula-Balak et al. [54] illustrated marked differences in the Leydig cell morphology after OP treatment. Mice Leydig cells exposed to experimental doses of OP (10^{-4} to 10^{-6} M) grew in a small group and 60% of cells showed nucleus shrinkage, cytoplasm vacuolization and membrane floating, while the control cells were formed as a monolayer with an epithelioid shape and abundant cytoplasm. Conversely, lower concentrations of OP did not markedly affect the morphological structure of exposed cells. In the recent years, a link was confirmed linking OP and the increased incidence of male reproductive dysfunction. The ability of OP to affect spermatogenesis has been the subject of much investigation. Spermatozoa abnormalities, a decreased sperm motility and lower spermatozoa viability are current problems mediated through OP exposition. Of the alkylphenols examined for their ability to act as an estrogen compound, octylphenol has been observed to be vastly effective, showing approx. one thousandth of the estrogenicity when compared to a strong estrogen 17β-estradiol [58]. Exposure to OP extremely inhibits the testicular function as exhibited by a reduced size of the testes, reduced androgen concentrations, and a negatively affected spermatogenesis. Similarities in the activity of OP and those noticed after the addition of 17β-estradiol indicate that OP exerted its effect to impair the testes in an estrogenic-like manner on the hypothalamus and/or anterior pituitary gland to arrest the gonadotropin secretion [59]. OP is also believed to support the reduction in sperm quantity in men resulting in male infertility and it has been defined as a potential reason of reproductive tumorigenesis [60]. It has also been reported that OP shows a toxic potency on cultured prespermatogonia and Sertoli cells [61]. In addition, it is proved that OP is able to generate ROS which are cytotoxic compounds resulting in oxidative damage associated with damage to biomolecules such as membrane lipids and DNA in sperm cells [62]. Adverse effects of OP on male reproductive functions in pubertal rats were evaluated by Herath et al. [63], when 50-day-old rats in the OP group received daily injections of the xenoestrogen at a concentration of 3 mg/kg. After 5 weeks of exposure, the epididymal sperm motility and sperm head counts were determined with reduced sperm counts resulting from a decreased plasma testosterone, but without significant effects of OP on the sperm motility parameters. The potential *in vivo* genotoxic activity of OP in adult male Wistar albino rats was studied by Ulutas et al. [64], when animals received OP oral doses of 125 and 250 mg/kg for 4 weeks. Possible genotoxic effects of OP were evaluated as comet parameters including tail length and tail moment with significant differences in both tested parameters only in the case of animals treated with the highest dose of OP. Peng et al. [65] also provide results of a combined genetic toxicity of OP along with NP in male mice following a peritoneal injection of nonylphenol-octylphenol (50,

100, and 200 mg/kg). The effect on the DNA damage in the testicular cells and sperm deformation rate after the exposure were measured using the comet assay and sperm morphologic test. Within the examined doses of 100 and 200 mg/kg, the quantity of the comet cells in the testes cells was increased. The DNA migration length was also significantly increased as OP-NP elevated and the rate of sperm deformation was higher following exposure to the tested chemicals too [66]. OP was also examined in the context of the biochemical composition of the seminal fluid and production of the viviparous eelpout (*Zoarces viviparus*) and the investigation was carried out at the time of spawning. After 10 days of exposure to OP, a decline in the gonadosomatic index was observed following the milt volume with a spermatocrit increase. The histological investigation manifested that OP impaired the lobular composition, including the Sertoli cells. In most of the OP-exposed individuals, trapped sperm cells in parts of the seminiferous lobules and the sperm ducts were observed. OP also affected the biochemical composition of the seminal fluid with elevated concentrations of the tested parameters such as magnesium, calcium, and total protein, meanwhile values of free amino acids were decreased in the exposed fish [67]. Movement characteristics are always the most important parameters in the evaluation of semen quality. Spermatozoa motility represents the primary characteristic in the assessment of male fertility and it is a fundamental premise for a successful fertilization. Motility parameters are closely linked to the mitochondrial activity of spermatozoa as these organelles play a key role in the energy provision by production of ATP [68]. Lukacova et al. [69] confirmed a decline of bovine sperm motility, progressive motility, and mitochondrial activity after exposure to 1–200 μg/mL OP during several time periods (0, 2, 4, and 6 h). Interestingly, the values of intracellular superoxide production revealed a slight decline of the superoxide concentration at the dose of 1 μg/mL when compared to the control group and conversely, doses 10, 100, and 200 μg/mL of OP increased the concentrations of superoxide in bovine sperm. Thus, in general, the effects of alkylphenols on the testicular function are not clearly defined yet and their effect may be attributed to the concentration, estrogen-mimicking activity, and time of exposure.

2.2. Bisphenols and their impact on steroidogenesis and spermatogenesis

Exposure to xenoestrogens such as bisphenols has been shown to cause adverse effects on male reproductive system in humans and numerous animal species. As typical endocrine disruptors, bisphenols are one of the most studied xenoestrogens in the field of male reproductive system. A survey of the Pubmed database provides more than 10,000 articles on the topic, including epidemiological as well as experimental studies. The overwhelming majority of bisphenols is used as stable components of household products, epoxy resins, inner surface of food metallic cans, dental sealants, and for myriad additional synthetic products. Many of us are mostly confronted by bisphenols through gastrointestinal exposure (food packaging) and dermal exposure (paper money and paper products). It is well known that increased concentration of bisphenols was detected in urine, milk or sweat and over 90% of human population is daily exposit to bisphenol A. Subsequent bioaccumulation and kinetic properties may adversely affect the overall health [70, 71]. Nowadays, bisphenols have been associated with a variety of human diseases, specifically kidney and cardiovascular diseases, obesity, developmental defects, and reproductive disorders. Recent studies indicate a direct link

between the incidence of male reproductive dysfunction and rising concentrations of bisphenols in the environment. A decrease in semen quality was the first reported alteration and from this moment on an informative expansion was launched on the potential consequences of bisphenol exposure [72]. Several reports demonstrate a direct effect of bisphenols on the biosynthesis of steroid hormones. Negative effects of bisphenol A (BPA) have been reported in both *in vivo* and *in vitro* studies, where the steroidogenic enzymes were recognized as primary targets. Downregulation of the expression levels of *CYP11A* and *CYP17A* has been observed primarily, resulting in the decline of testosterone synthesis [73]. The altered levels of testosterone may cause subsequent reproductive dysfunction by interfering with the feedback regulatory mechanisms. Another serious effect by which bisphenolic compounds perform their adverse impact on the male reproductive cells are disruption of the brittle balance between the antioxidant capacity of cells and prooxidants in testicular tissues, which is linked to the increased risk of oxidative stress development resulting in the arrest of spermatogenic processes, production of abnormal sperm cells, and impairment of normal existing sperm cells in the reproductive tract [11]. Oxidative reactions may lead to the decline of spermatozoa quality, as observed by the decrease of spermatozoa motility, velocity, and viability values. Moreover, bisphenol exposure could also result in the depletion of ATP metabolism and damage to the genetic material by sperm DNA fragmentation [74].

2.2.1. Bisphenol A

Lan et al. [75] evaluated the effects of BPA on two steroidogenic enzymes (*CP11A1; CYP19*) essential for the normal steroidogenic process. According to the PCR analysis, the endogenous gene expression in both was upregulated by BPA at 100–1000 nM. Another steroidogenic enzyme, *CYP17*, involved in the testosterone synthesis was also measured. The results showed that BPA did not affect *CYP17* protein expression significantly. However, the authors hypothesized that the balance of steroid hormones may be affected. This was confirmed in the next part of the study, where the testosterone production was slightly decreased at 1–100 nM BPA following a 24 h exposure. The next steroidogenic enzyme responsible for the conversion of pregnenolone to progesterone is 3β-HSD. Ye et al. [76] reported a significant inhibition of the 3β-HSD activity in rats and humans. Human 3β-HSD was more sensitive to BPA's inhibition than the rat enzyme. The authors also evaluated the effects of BPA on the testosterone production in rat Leydig cells. Experimental doses of 10 and 100 μM markedly decreased the testosterone generation. Importantly, evidence exists that exposure to BPA *in utero* may reduce the neonatal serum testosterone level [77]. In summary, although BPA directly affects the steroidogenic genes, it is clear that BPA disrupts the hormone synthesis and contributes to reproductive disorders. Because of an increased concern over the safety of BPA, European Union has banned its use in plastic bottles for infants. The viability of Leydig cells is a significant indicator for a sufficient production of steroid hormones. Lan et al. [75] illustrated a dose-dependent effect of BPA on this parameter in the MA-10 cell line. The data show a decrease in the cell viability (1–200 μM) following a 24 h cultivation *in vitro*. However, significant differences were recorded only with respect to the highest dose of BPA (200 μM). Goncalves's et al. [78] study showed a decrease in the Leydig cell viability upon the exposure to BPA. The authors found out that experimental doses above 1 μM inhibited the cell viability

following a 24 h incubation compared to the control. Nonetheless, the viability of TM3 cell line did not decrease significantly even after a 48 h exposure at concentrations below 50 μM. De Freitas et al. [79] observed a significant reduction in the viability of human Sertoli cells after the cultivation with 10 μM BPA for 48 h.

Nowadays, there are many epidemiological studies which evaluated the effect of bisphenols on the spermatozoa or spermatogenesis. Observable changes were recorded in the spermatozoa motility, spermatozoa viability, and DNA integrity. *In vivo* experiments with adult male rats indicated that the low concentration of BPA (2 μg/kg body weight) administered orally can effectively inhibit spermatogenesis via disruption of the biosynthesis of reproductive hormones resulting in the meiosis inhibition of sperm cells and induction of the Fas/FasL pathway with a subsequent apoptosis. Declining amounts of testosterone were followed by a reduction of sperm quantity [80]. Evidence showed an obvious link between increased urine levels of BPA and reduced values of the sperm concentration what can be attributed to the disturbed processes of spermatogenesis following BPA exposure. Harmful effects of BPA on the spermatogenesis observed in experimental animals are also in agreement with an epidemiological study focused on the impact of BPA on exposed human males. Reduced spermatozoa count, indicating a primary association between BPA exposure and production of sperm cells were attributed to increased values of BPA in urine when men with high urine BPA levels had more than three times lower sperm concentration and viability; however, no correlation was observed between the urine BPA concentrations and semen volume or abnormal sperm morphology compared to subjects without the presence of BPA in the urine [60]. Also, other *in vitro* studies revealed a direct effect of BPA exposure on the sperm quality. Singh et al. [81] used in his *in vitro* study chicken sperm to determine environmentally relevant concentrations of BPA (0.18, 0.37 and 0.74 mM) related to motility, fertilizing ability, live sperm percentage, and mitochondrial membrane potential after 30 min of BPA treatment. The results showed that 0.74 mM BPA is able to compromise sperm functions in the case of all analyzed parameters leading to the decline of sperm fertilizing ability. Data obtained from *in vitro* experiments by Lukacova et al. [82] refer that BPA has negative effects on bovine spermatozoa motility in different doses (1, 10, 100, and 200 μg/mL). The results showed that BPA has the ability to reduce the values of mitochondrial activity and spermatozoa motility, causing mitochondrial damage as evidenced by the increased values of intracellular superoxide. Spermatozoa motility parameters were significantly decreased in experimental groups exposed to concentrations of BPA higher than 100 μg/mL. In experimental mice, the motility analyzed following 6 h of *in vitro* treatment with 0.0001, 0.01, 1, and 100 μM BPA, the number of motile sperm cells was also reduced in the case of dose of 100 μM BPA [83]. Administration of different BPA concentrations (0.6, 4.5, and 11.0 μg/L) demonstrated an impairment of motility in fish spermatozoa too [84].

2.2.2. Bisphenol alternatives

More stringent global regulations of BPA production and the use have led to the development of alternative bisphenol compounds [85]. A few years ago, researchers have begun to deal with potential properties of 4,4′-dihydroxydiphenylsulphone (BPS) or 4,4′-dihydroxydiphenlymethane (BPF). Both are presently not regulated and are used without restriction. Additionally,

currently available toxicological data are scarce and the information about their potential impact is limited. Nowadays, studies reported the effects of BPS via genomic mechanisms using extremely high concentrations but there are still no studies evaluating the *in vivo* toxicity. Although BPS is less likely to leach from plastic packaging with heat, it does still escape the polymer in small quantities under the normal use. Chen et al. [86] showed that 40 μM BPS had a 15-fold lower genomic estrogenic activity than BPA. Only a few studies have evaluated BPS at low concentration ranges likely to be present in foods, wildlife or humans. Eladak et al. [87] used the mouse FeTA model to illustrate the effects of BPS and BPF on the testosterone synthesis. Results from the present study showed that BPF has a similar dose-response effect as BPA with a significantly decreased amount of testosterone starting from 1000 nmol/L. On the other hand, BPS had even a more potent inhibitory effect than BPA. Indeed, 100 nmol/L BPS significantly reduced the testosterone production after 3 days of treatment. Authors also compared the effects of 10,000 nmol/L BPA, BPS, and BPF on specific gene expression in mice Leydig cells. All bisphenol alternatives reduced the expression of key genes involved in steroidogenesis such as *Star, hsd3βa CYP17a1*, expect *CYP11a1*. In addition, the expression of Lhcgr (the gene encoding the LH/CG receptor) was also decreased. This is one of the few reports that suggest harmful consequences on the reproductive functions in humans and rodents. According to Ji et al. [88], BPS is able to reduce the level of testosterone as well as *CYP17*a and *17β-HSD* mRNA levels in zebrafish. It must be noted that the binding activity of BPS and BPF to estrogen receptor (α; β) is, respectively, 5-or 10-fold lower than that of BPA in the HELN cells [89].

Effect of BPS exposure on oxidative stress, generation of ROS, and impairment of DNA integrity of rat sperm cells under the *in vitro* condition and daily sperm production and sperm DNA damage under the *in vivo* condition was examined in the study of Ullah et al. [90]. Spermatozoa were cultivated along with BPS at doses of 0.5, 1, 10 and 100 μg/L and the analyses showed that the highest concentration of BPS initiated ROS generation, induced peroxidation of membrane lipids, altered superoxide dismutase concentrations, and increased the incidence of DNA fragmentation in the sperm cells. The *in vivo* part of this study revealed that adult rats exposed to concentrations of 0.5, 5, 25, and 50 μg/kg/day for 28 days demonstrated a decline in daily sperm production with rising values of DNA damage occurring in spermatozoa observed in experimental animals treated with the highest dose (50 μg/kg/day) of BPS; however, the motility parameters were not inhibited. Similarly, treatment with 50 μg/kg/d lead to the development of oxidative stress in the testes and impaired reproductive functions in rats [91]. An earlier study on zebrafish embryos focusing on the developmental exposure to BPS was performed to examine the reproduction potential and hormonal balance in adult individuals. Embryos of zebrafish were treated and bred in the presence of various doses of BPS (0, 0.1, 1, 10, and 100 g/L) for 75 days. Following that period, adult males and females were paired for next 7 days in fresh water and subsequently the impact on individual development, reproduction, plasma vitellogenin, sex steroids, and thyroid hormone rates were examined. The results showed skewed sex ratio in favor of females and decreased values of body length and weight in males exposed to 100 g/L of BPS. The gonadosomatic index showed reduced values in fish at tested concentrations ≥10 g/L of BPS. In both males and females, a significant stimulation in plasma vitellogenin level was noticed at ≥10 μg/L of BPS and also thyroxine and

triiodothyronine levels were significantly decreased at 10 and 100 μg/L of BPS in males. Sperm count was also reduced in the experimental groups exposed to 10 and 100 μg/L of BPS [92]. In other studies, cytotoxic, genotoxic [93], and mutagenic [94] effects of BPS in different cell models were documented. It is proved that the exposure to BPS can violate the cellular signaling path in the apoptotic and viability ways, which is why it is possible to expect a reaction of BPS with pro-apoptotic and signaling cascades observed also in the sex cells resulting in the affected cell cycle and apoptosis [95]. Nowadays, further research is required to elucidate the effects of bisphenols on the male and female reproductive system.

2.3. Phthalates and their impact on steroidogenesis and spermatogenesis

Numerous environmental contaminants have hormonal or anti-hormonal actions that interfere with endocrine homeostasis of individuals. As we mentioned above, the group of endocrine disruptors is very heterogeneous and phthalates, as ubiquitous chemical compounds are widely used as plasticizers in children's plastics toys, food packaging, medical tubing, certain cosmetics, shampoos, soaps, and many others household products [96]. Early experimental studies found a low level of phthalate toxicity in rodents, but nowadays, a high extent of carcinogenicity, teratogenicity or testicular atrophy has been widely confirmed. Recent studies have verified that phthalates are capable to affect many physiological mechanism and functions, especially within the reproductive system. Moreover, disorders linked to reproductive toxicity may appear in early life stages, puberty, and some of them may manifest in adulthood. The Department of Health and Human Services estimated that daily human consumption of commonly used phthalates diethylhexyl phthalate (DEHP) revolves around 5.8 mg and monoethylhexyl phthalate (MEHP) ranges from 3.26 to 4.15 in males and 2.93 to 3.51 in females. On the other hand, DEHP is metabolized by intestinal lipases to MEHP, which is glucuronized and excreted from the organism with minimum tissue accumulation [97, 98]. According to its toxicological profile, MEHP seems to be 10-fold more potent in its toxicity to Leydig and Sertoli cells in comparison to DEHP, suggesting that DEHP is the pretoxin which acts via metabolizing into MEHP [99]. Several toxicological reports suggest that DEHP and MEHP disrupt reproductive development and now it is established that these phthalates inhibit the biosynthesis of steroid hormones in Leydig cells at different developmental stages. *In utero* exposure to phthalates has been shown to reduce male fertility potential in rats. Subsequent postnatal changes preceded an inhibition in Leydig cell function, including lower levels of testosterone. Many authors suggest that phthalates exert their effect via multiple mechanism of action such as the peroxisomes proliferator-activated receptors, estrogen receptors or yet unidentified mechanism.

2.3.1. Diethylhexyl phthalate (DEHP)

Akingbemi et al. [100] investigated the ability of DEHP to affect the biosynthesis of steroid hormones in rat Leydig cells. Pubertal rats were exposed to 1, 10, 100, and 200 mg/kg/day DEHP for 2 weeks. The highest experimental dose (200 mg/kg/day) DEHP caused a 77% decrease in the activity of 17β-HSD and reduced the testosterone production to 50% of the control. Paradoxically, prolonged time of cultivation to 28 days resulted in significant increases in the testosterone secretion capacity and in serum LH levels. A few years later, Akingbemi et al. [101] evaluated the

potential effects of DEHP on isolated rat Leydig cells *in vitro*. When compared to the control, mRNA levels of PCNA and cyclin D3 were expressed at statistically higher levels of proliferation following treatment. Additionally, estradiol levels were elevated by as much as 50% above the control group and aromatase gene expression was also higher in DEHP exposed cells. Several recent investigations have shown that DEHP disrupts the reproductive system of the male rat in an antiandrogenic manner. In the present study, Parks et al. [102] explored the antiandrogenic action of DEHP and MEHP as well as alterations in the testosterone production. Maternal exposure at 750 mg/kg/day caused a significant reduction in the testosterone levels. In addition, Liu et al. [103] performed gene expression profiling following *in utero* exposure to phthalates and observed a decline in levels of steroidogenic enzymes (*CYP11a1*; *CYP17a1*) and lipid transport (StAR). However, the exact mechanism of action is not fully clear. The negative impact of DEHP on the male reproductive system has been related to their monoester metabolite MEHP. It has been shown that this endocrinologically active phthalate may negatively affect the testes and more specifically suppress Leydig cells functions [104].

2.3.2. Monoethylhexyl phthalate (MEHP)

Dees et al. [105] reported that MEHP inhibits androgen production in MA-10 Leydig cells. By using different MEHP concentrations over a longer time interval (24 and 2 h), the authors have demonstrated that even at low experimental doses MEHP inhibits the steroid production (a 50% inhibition was observed at 10 μM), induces morphological changes such as mitochondrial swelling and vesiculation of the Golgi apparatus. Conversely, at 100 and 300 μM doses, this inhibition was not seen. Thus, it is possible that the absence of any effect may be mediated through an unidentified mechanism, distinct to the mechanisms responsible for the inhibition of steroid production. In the next *in vitro* study, Jones et al. [106] exposed the primary culture of Leydig cells to MEHP (1 mM) for 2 h. A moderate decrease in testosterone production was shown which correlated with the changes in the cell ultrastructure. Treatment with MEHP confirmed mitochondrial swelling with the loss of matrix granules, reduction in the number of Golgi apparatus and dilatation of the smooth endoplasmic reticulum. Svechnikov et al. [107] also confirmed the inhibitory effect on steroidogenesis in rat Leydig cells. The result showed significantly lower testosterone levels (57–62% inhibition) in exposed cells (250 μM MEHP) after 24 h incubation when compared with the control group. In order to determine whether the inhibition of testosterone secretion was due to the disruption of StAR, the authors decided to monitor the expression of this protein by Western blotting. A marked decrease in StAR expression was observed after 24 h incubation. In addition, the activity of 5α-reductase, an enzyme synthesizing the potent androgen dihydrotestosterone, was dramatically inhibited in immature Leydig cells. The dysfunction of Leydig cells is postulated to have a direct association with androgen-dependent parameters of sexual development. Nevertheless, it is necessary to determine whether the effects of chronic DEPH or MEHP exposure are reversed or mitigated when exposure is terminated.

Numerous studies have evaluated the testicular toxicity of phthalates in different experimental models and showed that spermatozoa and spermatogenesis were one of the main targets of their actions. Kasahara et al. [96] indicate associations between DEHP administration and increased production of ROS and selectively decreased GSH and ascorbic acid in the testis with a consequent induction of rat sperm cell apoptosis leading to testicular atrophy after *in vivo* DEHP exposition. More specifically, the results provided by Li et al. [108] when male rats were fed

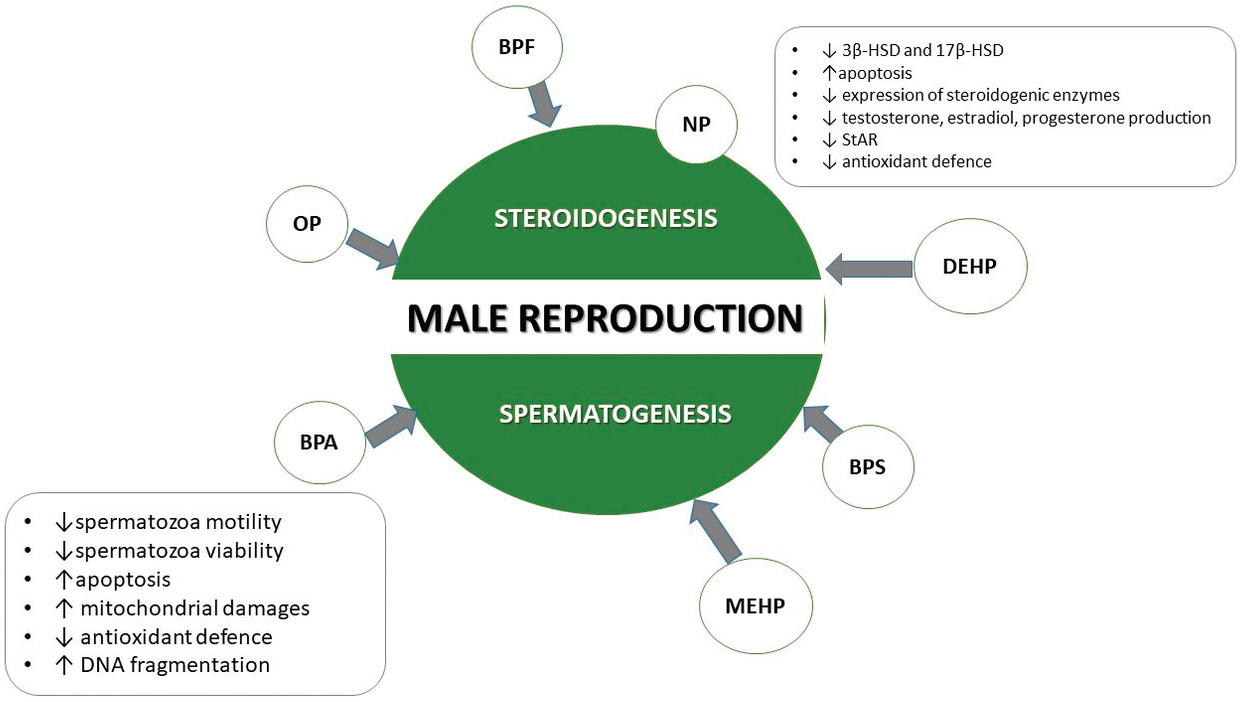

Figure 1. The effects of selected EDs on the male reproductive system.

DEHP for 2 weeks. The result was that the spermatogenesis became disrupted with decreased spermatocytes and spermatids counts and in addition, DEHP (20, 100, 500, to 1000 mg/kg) appeared to inhibit DNA replication. This resulted in the induction of the mitochondrial apoptotic pathways and overgeneration of ROS. Also, elevated activity of superoxide dismutase, reduced activity of glutathione peroxidase, and increased values of malondialdehyde after exposure to 500 mg/kg/day of dibutyl phthalate in the epididymis support the importance of oxidative stress as a major mechanism of phthalate action [109]. Likewise, the response to oxidative stress indicates an increased expression of mitochondrial peroxiredoxin and cyclooxygenase-2 in germ cells after phthalate treatment [110]. Apoptosis of germ cells has also been proposed as a potential effect of phthalates on male reproduction based on the results reporting an increased membrane localization of Fas and apoptic cells [111, 112]. One essential trace element necessary in spermatogenesis is zinc and even a slight deficiency of zinc has been observed to arrest spermatogenesis in both mice and humans [113]. Earlier studies examined phthalate-induced modifications in metabolism of zinc after treatment with high doses of phthalates with reduced testicular zinc concentrations [114], a decline of zinc half-life in the testes [115] and increased excretion of zinc in urine after phthalate exposure [114, 116]. The next schematic figure **(Figure 1)** summarizes final findings.

3. Future directions and recommendations

Probably, research is just at the very start of a long journey to refine understanding of the principal mechanisms of toxicity related to endocrine disruptive compounds and the range of influence of these hormonally active substances to the human and environmental health in the

context of male reproduction. Society will definitely continue to use these materials because of their undeniable benefits and primary we have to aim future investigation on testing and development of chemicals to maintain healthier, safe, and more sustainable world for next generations and on evolve suitable strategies of remediation of EDs. Progress in the experimental area of endocrine disruptors effects provides rich lessons that can be usable in other fields of science, as well as in the future missions in toxicology and environmental health.

This still controversial and live topic has already improved research of toxicology and risk assessment and has moved it into certain radically different trends. Further improvement in this field including reproductive biology rests in modern technology, such as toxicogenomics, which can study precursor changes on the level of cells and biological molecules and thus offer understanding of dose and time-dependent responses in more detail. Moreover, the increased usage of human, rather than animal, cell models keep a promise for intensify issues of human relevance. However, reality is that new questions are asked while previous issues associated with impact of EDs on male reproductive organs and behavior persist. The most important fields of investigation for better understanding of how EDs affect functions of tissues involved in male reproductive physiology are associated especially with questions such as why are some tissues, time periods, and even organisms more resistant to EDs exposure; how EDs effect in model organisms and cells translates to human exposure to EDs. There is also need for more studies with aim on syndromes and EDs contribution to development of multiple symptoms at once. The summary of some EDs affecting male reproductive system is presented in **Table 1**. There is also necessity to interpret specific cell culture responses in the context of whole-organism physiology, ideally that of humans. It is well known that endocrine system

Chemicals	Cellular effects	Source/applications	Study
Aldrin	Competitive binding to androgen receptors; ↓weight of testes; ↓ 3β-HSD and 17β-HSD; ↓spermatozoa MOT;	Insecticide, groundwater	Lemaire et al. [117] Chatterjee et al. [118] Das Neves et al. [119]
Alachlor	Competitive binding to estrogen and progesterone receptors; no effects on testosterone production; ↓ spermatozoa MOT and viability;	Herbicide	Mikamo et al. [120] Gizard et al. [121]
Bisphenols	Estrogenic and anti-androgenic affinity; ↓ 3β-HSD and 17β-HSD; ↑apoptosis; ↓ sperm MOT, viability and concentration;	Plasticizers, epoxy resins, dental sealants,	Eladak et al. [87] Lukacova et al. [82] Akingbemi et al. [122] Ahmed [123]
DDT and metabolites	Competitive binding to androgen receptors, activation of androgen-sensitive cells proliferation; ↓ expression of steroidogenic enzymes; ↓ testosterone, estradiol, progesterone production;	Pesticides, insecticide	Tapiero et al. [124] Tesier and Matsumura [125] Castellanos et al. [126]

Chemicals	Cellular effects	Source/applications	Study
Mono/Di-(2-ethylhexyl) phthalate	↓17β-HSD; ↓ StAR expression, ↑ mitochondrial damages; ↑ ROS; ↓ antioxidant defense; ↑spermatozoa apoptosis;	Plasticizers, cosmetics, food packaging	Akingbemi et al. [100, 101] Svechnikov et al. [107] Dees et al. [105]
Alkylphenols	↓3β-HSD, 17β-HSD, StAR; ↑ ROS production; ↓ cell viability; ↑ apoptosis; ↓ spermatozoa MOT and viability; ↑ DNA fragmentation,	Cosmetics, pesticides, paints, food packaging's,	Jambor et al. [21, 57] Lukacova et al. [69, 82] Diemer et al. [127] Haavisto et al. [128]

3β-HSD, 3beta-hydroxysteroid dehydrogenase; 17β-HSD, 17beta-hydroxysteroid dehydrogenase; MOT, motility; StAR, steroidogenic acute regulatory protein; and ROS, reactive oxygen species.

Table 1. Summary of some EDs affecting male reproduction.

mediates reactions on distant tissues and cells. Therefore, research that focuses only on isolated components of endocrine system or target tissues may provide incomplete information. Essential principles of toxicokinetics should be part of key studies related to impact of EDs on specific structures of organisms.

4. Conclusion

In recent years, a growing incidence of EDs has led scientific community to show how these substances may affect the male reproductive system. The *in vitro* evaluation of steroidogenesis and spermatogenesis are necessary for the screening potential of reproductive toxicants such as alkylphenols, bisphenols, phthalates, and many others. The mechanism of their negative effect is by diverse but one important endpoint is reduced processes, essential for normal reproductive functions. This review has demonstrated that certain groups of EDs may directly or indirectly interfere with the biosynthesis of steroid hormones and spermatogenesis via different mechanisms of action. Dysfunction of these processes may cause an incomplete masculinization, suppressed libido, reduced steroidogenic capacity, develop various malformations in spermatozoa and subsequently totally inhibit the reproductive potential of humans and animals. It must be noted that further studies are required to understand the effects of EDs on the male reproductive functions and their contributions to male sub- or infertility.

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Conflict of interest

The authors declare no conflicts of interest.

Abbreviations

AR	androgen receptor
AhR	aryl hydrocarbon receptor
cAMP	cyclic adenosine monophosphate
ER	estrogen receptor
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
ROS	reactive oxygen species

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References

[1] Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M, Skakkebaek NE, Toppari J. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet. 2004;**363**:1264-1269. DOI: 10.1016/s0140-6736(04)15998-9

[2] Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: Potential need and demand for infertility medical care. Human Reproduction. 2007;**22**:1506-1512. DOI: 10.1093/humrep/dem046

[3] Baladanič D, Rupnik M, Klemančič AK. Negative impact of endocrine-disrupting compounds on human reproductive health. Reproduction, Fertility, and Development. 2011; **23**:403-416. DOI: 10.1071/RD09300

[4] Tyler CR, Jobling S, Sumpter JP. Endocrine disruption in wildlife: A critical review of the evidence. Critical Review in Toxicology. 1998;**28**:319-361. DOI: 10.1080/10408449891344236

[5] Sanderson JT. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. Toxicological Sciences. 2006;**94**:3-21. DOI: 10.1093/toxsci/kfl051

[6] Svechnikov K, Landreh L, Weisser J, Izzo G, Colón E, Svechnikova I, Soder O. Origin, development and regulation of human Leydig cells. Hormone Research in Pædiatrics. 2010;**73**:93-101. DOI: 10.1159/000277141

[7] Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. Archives of Environmental Contamination and Toxicology. 2002; **43**:121-126. DOI: 10.1007/s00244-001-0048-8

[8] Wagner M, Oehlmann J. Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-screen. The Journal of Steroid Biochemistry and Molecular Biology. 2011;**127**: 128-135. DOI: 10.1016/j.jsbmb.2010.10.007

[9] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: An Endocrine Society scientific statement. Endocrine Reviews. 2009;**30**:293-342. DOI: 10.1212/er.2009-0002

[10] Wolff MS. Occupational exposure to polychlorinated biphenyls (PCBs). Environmental Health Perspectives. 1985;**60**:133-138. DOI: 10.1289/ehp.8560133

[11] Mathur PP, D'Cruz SC. The effect of environmental contaminants on testicular function. Asian Journal of Andrology. 2011;**13**:585-591. DOI: 10.1038/aja.2011.40

[12] Berman T, Levine H, Gamzu R, Grotto I. Trends in reproductive health in Israel: Implications for environmental health policy. Israel Journal of Health Policy Research. 2012;**34**:1-8. DOI: 10.1186/2045-4015-1-34

[13] Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology. 2006;**17**:682-691. DOI: 10.1097/01.ede.0000235996.89953.d7

[14] Meekr JD, Ehrlich S, Tooth TL, Wright DL, Calafat AM, Trisini AT, Ye X, Hauser R. Semen quality and sperm DNA damage in relation to urinary bisphenol a among men from an infertility clinic. Reproductive Toxicology. 2010;**30**:535-539. DOI: 10.1016/j.teprotox.2010.07.005

[15] Nimrod AC, Benson WH. Environmental estrogenic effects of alkylphenol ethoxylates. Critical Reviews in Toxicology. 1996;**26**:335-364. DOI: 10.3109/1048449609012527

[16] Klecka GM, Staples CA, Naylor CG, Woodburn KB, Losey BS. C8- and C9-alkylphenols and ethoxylates: II. Assessment of environmental persistence and bioaccumulation potential. Human and Ecological Risk Assessment: An International Journal. 2008;**16**: 1025-1055. DOI: 10.1080/10807030802387747

[17] Andersen HR, Vinggaard AM, Rasmussen TH, Gjermandsen IM, Bonefeld-Jorgensen EC. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and

aromatase activity in vitro. Toxicology and Applied Pharmacology. 2002;**179**. DOI: 1-12. DOI:10.1006/taap.2001.9347

[18] Vazquez-Duhalt R, Marquez-Rocha F, Rivas EP, Licea A, Viana MT. Nonylphenol, and integrated vision of a pollutant. Scientific review. Applied Ecology and Environmental Research. 2005;**4**. DOI: 1-25. DOI:10.15666/aeer/0401_001025

[19] Ying F, Ding CH, Ge R, Wang X, Li F, Zhang Y, Zeng Q, Yu B, Ji R, Han X. Comparative evaluation of nonylphenol isomers on steroidogenesis of rat Leydig cells. Toxicology In Vitro. 2012;**26**:1114-1121. DOI: 10.1016/j.tiv.2012.06.016

[20] Wu JJ, Wang KL, Wang SW, Hwang IM, Chen ML, Wang PS. Differential effects of nonylphenol on testosterone secretion in rat Leydig cells. Toxicology. 2010;**268**:1-7. DOI: 10.1016/j.tox.2009.10.030

[21] Jambor T, Tvrdá E, Tušímová E, Kováčik A, Bistáková J, Forgács Z, Lukáč N. In vitro effect of 4-nonylphenol on human chorionic gonadotropin (hCG) stimulated hormone secretion, cell viability and reactive oxygen species generation in mice Leydig cells. Environmental Pollution. 2017;**222**:219-225. DOI: 10.1016/j.envpol.2016.12.053

[22] Majdic G, Sharpe RM, PJ O'S, Saunders PT. Expression of cytochrome P450 17alpha-hadroxylase/C17-20 lyase in the fetal rat testis is reduced by maternal exposure to exogenous estrogens. Endocrinology. 1996;**137**:1063-1070. DOI: 10.1210/endo.137.3.8603575

[23] Miura C, Takahashi N, Michino F, Miura T. The effect of Para-nonylphenol on Japanese eel (*Anguilla japonica*) spermatogenesis *in vitro*. Aquatic Toxicology. 2005;**71**:133-141. DOI: 10.1016/j.aquatox.2004.10.015

[24] Wang X, Han X, Hou Y, Yao G, Wang Y. Effect of nonylphenol on apoptosis of Sertoli cells in vitro. Bulletin of Environmental Contamination and Toxicology. 2003;**70**:898-904. DOI: 10.1007/s00128-003-0067-4

[25] Han XD, Tu GZ, Gong Y, Shen SN, Wang XY, Kang LN, Hou YY, Chen JX. The toxic effects of nonylphenol on the reproductive system of male rats. Reproductive Toxicology. 2004;**19**:215-221. DOI: 10.1016/j.reprotox.2004.06.014

[26] Gong Y, Wu J, Huang Y, Shen S, Han X. Nonylphenol induces apoptosis in rat testicular Sertoli cells via endoplasmic reticulum stress. Toxicology Letters. 2009;**186**:84-95. DOI: 10.1016/j.toxlet.2009.01.010

[27] Tan IP, Roy C, Sáez JC, Paul DL, Risley MS. Regulated assembly of connexin 33 and connexin 43 into rat Sertoli cell gap junctions. Biology of Reproduction. 1996;**54**:1300-1310. DOI: 10.1095/biolreprod54.6.1300

[28] Pérez-Armendariz EM, Lamoyi E, Mason JI, Cisneros-Armas D, Luu-The V, Moreno B, JF. Developmental regulation of connexin 43 expression in fetal mouse testicular cells. The Anatomical Record. 2001;**264**:237-246. DOI: 10.1002/ar.1164

[29] Aravindakshan J, Cyr DG. Nonylphenol alters connexin 43 levels and connexin 43 phosphorylation via an inhibition of the p38-mitogen-activated protein kinase pathway. Biology of Reproduction. 2005;**72**:1323-1240. DOI: 10.1095/biolreprod.104.038596

[30] Lee PC, Arndt P, Nickels KC. Testicular abnormalities in male rats after lactational exposure to nonylphenols. Endocrine. 1999;**11**:61-68. DOI: 10.1385/ENDO:11:1:61

[31] Hossaini A, Dalgaard M, Vinggaard AM, Frandsen H, Larsen JJ. In utero reproductive study in rats exposed to nonylphenol. Reproductive Toxicology. 2001;**15**:537-543. DOI: 10.1016/s0890-6238(01)00155-1

[32] Schiffer C, Muller A, Egeberg DL, Alvarez L, Brenker C, Rehfeld A, Frederiksen H, Waschle B, Kaupp UB, Balbach M, Wachten D, Skakkebaek NE, Almstrup K, Strunker T. Direct action of endocrine disrupting chemicals on human sperm. EMBO Reports. 2014; **15**:758-765. DOI: 10.15252/embr.201438869

[33] Tohyama S, Miyagawa S, Lange A, Ogino Y, Mizutani T, Tatarazako N, Katsu Y, Ihara M, Tanaka H, Ishibashi H, Kobayashi T, Tyler CR, Iguchi T. Understanding the molecular basis for differences in responses of fish estrogen receptor subtypes to environmental estrogens. Environmental Science and Technology. 2015;**49**:7439-7447. DOI: 10.1021/acs.est.5b00704

[34] Shao ZX, Jiang HT, Liang F, Zhu BC. Effects of nonylphenol and cadmium on sperm acrosome reaction in vitro in mice. Zhonghua Nan Ke Xue. 2011;**17**:318-321

[35] Uguz C, Varisli O, Agca C, Evans T, Agca Y. *In vitro* effects of nonylphenol on motility, mitochondrial, acrosomal and chromatin integrity of ram and boar spermatozoa. Andrologia. 2015;**47**:910-919. DOI: 10.1111/and.12346

[36] Jager C, Bornman MS, Oosthuizen JMC. The effect of p-nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. Andrologia. 2009;**31**:107-113. DOI: 10.1111/j.1439-0272.1999.tb02853.x

[37] Aly HA, Domenech O, Banjar ZM. Effect of nonylphenol on male reproduction: Analysis of rat epididymal biochemical markers and antioxidant defense enzymes. Toxicology and Applied Pharmacology. 2012;**261**:134-141. DOI: 10.1016/j.taap.2012.02.015

[38] Ye XF, Yao YF, Wang LZ. Study on reproductive toxicity in male embryo rats with the pregnancy SD rates exposed by nonylphenol. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2012;**30**:856-858

[39] Huang W, Quan C, Duan P, Tang S, Chen W, Yang K. Nonylphenol induced apoptosis and autophagy involving the Akt/mTOR pathway in prepubertal Sprague-Dawley male rats in vivo and in vitro. Toxicology. 2016;**373**:41-53. DOI: 10.1016/j.tox.2016.11.006

[40] Uguz C, Varisli O, Agca C, Agca Y. Effects of nonylphenol on motility and subcellular elements of epididymal rat sperm. Reproductive Toxicology. 2009;**28**:542-549. DOI: 10.1016/j.reprotox.2009.06.007

[41] Lukac N, Lukacova J, Pinto B, Knazicka Z, Tvrda E, Massanyi P. The effect of nonylphenol on the motility and viability of bovine spermatozoa *in vitro*. Journal of Environmental Science and Health A. 2012;**48**:973-979. DOI: 10.1080/10934529.2013.762744

[42] Uguz C, Varisli O, Agca C, Agca Y. Effects of nonylphenol on motion kinetics, acrosome and mitochondrial membrane potential in frozen-thawed bull sperm. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2014;**20**:583-590. DOI: 10.9775/kvfd.2014.10459

[43] Ergun SS, Ustuner B, Alcay S, Sagirkaya H, Uguz C. The effects of nonylphenol on gamete physiology in bovine. Journal of Applied Biological Science. 2014;**8**:32-38

[44] Toft G, Baatrup E. Sexual characteristics are altered by 4-tert-Octylphenol and 17b-estradiol in the adult male guppy (*Poecilia reticulata*). Ecotoxicology and Environmental Safety. 2001;**48**:76-84. DOI: 10.1006/eesa.2000.1985

[45] Feng M, Peng C, Xue W, Yingmei Z, Wenya Z, Yongmei Q. Effect of 4-nonylphenol on the sperm dynamic parameters, morphology and fertilization rate of Bufo raddei. African Journal of Biotechnology. 2011;**10**:2698-2707. DOI: 10.5897/ajb10.1974

[46] Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, Brandt I, Vethaak AD. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the european situation. Critical Reviews in Toxicology. 2000;**30**:71-133. DOI: 10.1080/10408440091159176

[47] McLachlan JA. Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals. Endocrine Reviews. 2001;**22**:319-341. DOI: 10.1210/er.22.3.319

[48] Tsuda T, Takino A, Muraki K, Harada H, Kojima M. Evaluation of 4-nonylphenols and 4-tert- octylphenol contamination of fish in rivers by laboratory accumulation and excretion experiments. Water Research. 2001;**35**:1786-1792. DOI: 10.1016/s0043-1354(00)00445-0

[49] Quirós L, Céspedes R, Lacorte S, Viana P, Raldúa D, Barceló D, Piña B. Detection and evaluation of endocrine-disruption activity in water samples form Portuguese rivers. Environmental Toxicology and Chemistry. 2005;**24**. DOI: 389-395. DOI:10.1897/07-121r.1

[50] Qian J, Bian Q, Cui L, Chen J, Song L, Wang X. Octylphenol induces apoptosis in cultured rat Sertoli cells. Toxicology Letters. 2006;**166**:178-186. DOI: 10.1016/j.toxlet.2006.06.646

[51] Murono EP, Derk RC. Exposure to octylphenol increases basal testosterone formation by cultured rad Leydig cells. The Journal of Steroid Biochemistry and Molecular Biology. 2002;**81**:181-189. DOI: 10.1016/s0960-0760(02)00054-7

[52] Hanukoglu I, Rapoport R, Weiner L, Sklan D. Electron leakage from the mitochondrial NADPH-adrenodoxin reductase-adrenodoxin-P450scc (cholesterol side chain cleavage) system. Archives of Biochemistry and Biophysics. 1993;**305**:489-498. DOI: 10.1006/abbi.1993.1452

[53] Nikula H, Talonpoinka T, Kaleva M, Toppari J. Inhibition of hCG-stimulated steroidogenesis in cultured mouse Leydig tumor cells by bisphenol a and octylphenols. Toxicology and Applied Pharmacology. 1999;**157**:166-173. DOI: 10.1006/taap.1999.8674

[54] Kotula-Balak M, Pochec E, Hejmej A, Duda M, Bilinska B. Octylphenol affects morphology and steroidogenesis in mouse tumor Leydig cells. Toxicology In Vitro. 2011;**25**:1018-1026. DOI: 10.1016/j.tiv.2011.03.021

[55] Murono EP, Derk RC, DeLeón JH. Biphasic effects of octylphenol on testosterone biosynthesis by cultured Leydig cells from neonatal rats. Reproductive Toxicology. 1999;**13**:451-462. DOI: 10.1016/S0890-6238(99)00047-7

[56] Majdic G, Sharpe RM, Saunders PTK. Maternal oestrogen/xenoestrogen exposure alters expression of steroidogenic factor-1 (SF1/ad4BP) in the fetal rat testis. Molecular and Cellular Endocrinology. 1997;**127**:91-987. DOI: 10.1016/S0303-7207(96)03998-6

[57] Jambor T, Tvrdá E, Bisáková J, Forgács Z, Lukáč N. The potential impact of 4-octylphenol on the basal and stimulated testosterone formation by isolated mice Leydig cells. Journal of Central European Agriculture. 2016;**17**:1274-1286. DOI: 10.5513/JCEA01/17.4.1844

[58] White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology. 1994;**135**:175-182. DOI: 10.1210/en.135.1.175

[59] Blake CA, Boockfor FR. Chronic administration of the environmental pollutant 4-tert-octylphenol to adult male rats interferes with the secretion of luteinizing hormone, follicle-stimulating hormone, prolactin, and testosterone. Biology of Reproduction. 1997;**57**:255-266. DOI: 10.1095/biolreprod57.2.255

[60] Li E, Guo Y, Ning Q, Zhang S, Li D. Research for the effect of octylphenol on spermatogenesis and proteomic analysis in octylphenol-treated mice testes. Cell Biology International. 2011;**35**:305-309. DOI: 10.1042/cbi20100566

[61] Raychoudhury SS, Blake CA, Millette CF. Toxic effects of octylphenol on cultured rat spermatogenic cells and sertoli cells. Toxicology and Applied Pharmacology. 1999;**157**:192-202. DOI: 10.1006/taap.1999.8664

[62] Kabuto H, Hasuike S, Minagawa N, Shishibori T. Effects of bisphenol a on the active oxygen species in mouse tissues. Environmental Research. 2003;**93**:31-35. DOI: 10.1016/s0013-9351(03)00062-8

[63] Herath CB, Jin W, Watanabe G, Arai K, Suzuki AK, Taya K. Adverse effects of environmental toxicants, octylphenol and bisphenol a, on male reproductive functions in pubertal rats. Endocrine. 2004;**25**:163-172. DOI: 10.1385/endo:25:2:163

[64] Ulutas OK, Yildiz N, Durmaz E, Ahbab MA, Barlas N, Cok I. An in vivo assessment of the genotoxic potential of bisphenol a and 4-tert-octylphenol in rats. Archives of Toxicology. 2011;**85**:995-1001. DOI: 10.1007/s00204-010-0620-y

[65] Peng Y, Jian Z, Yuan-bin Y, Dan-yang Z, Jing-ye X. Study of nonylphenol and octylphenol on combined genetic toxicity of mice. Chinese Journal of Health Laboratory Technology. 2008;**12**

[66] Sweeney T, Fox J, Robertson L, Kelly G, Duffy P, Lonergan P, O'Doherty J, Roche JF, Evans NP. Postnatal exposure to octylphenol decreases semen quality in the adult ram. Theriogenology. 2007;**67**:1068-1075. DOI: 10.1016/j.theriogenology.2006.12.010

[67] Rasmussen TH, Korsgaard B. Estrogenic octylphenol affects seminal fluid production and its biochemical composition of eelpout (*Zoarces viviparus*). Comparative Biochemistry and Physiology C. 2004;**139**:1-10. DOI: 10.1016/j.cca.2004.08.016

[68] Martinez C, Mar C, Azcarate M, Pascual P, Aritzeta JM, Lopez-Urrutia A. Sperm motility index: A quick screening parameter from sperm quality analyser-IIB to rule out oligo- and asthenozoospermia in male fertility study. Human Reproduction. 2000;**15**:1727-1733. DOI: 10.1093/humrep/15.8.1727

[69] Lukacova J, Tvrda E, Lukac N. The effect of an in vitro exposure to octylphenol on bovine spermatozoa. Animal welfare. Ethology and Housing Systems. 2013;**9**:558-566

[70] Vandenberg LN, Hauser R, Marcus M, Olea N, Wlshons WV. Human exposure to bisphenol a (BPA). Reproductive Toxicology. 2007;**24**:139-177. DOI: 10.1016/j.reprotox.2007.07.010

[71] Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol a and 4-tertiary-octylphenol:2003–2004. Environmental Health Perspectives. 2008;**116**:39-44. DOI: 10.1289/ehp.10753

[72] Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. British Medical Association. 1992;**305**:609-613. DOI: 10.1097/00006254-199303000-00023

[73] Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine Reviews. 2001;**32**:81-151. DOI: 10.1210/er.2010-0013

[74] Hulak M, Gazo I, Shaliutina A, Linhartova P. *In vitro* effects of BPA on the quality parameters, oxidative stress, DNA integrity and adenosine triphosphate content in sterlet (*Acipenser ruthenus*) spermatozoa. Comparative Biochemistry and Physiology. 2013;**158**:64-71. DOI: 10.1016/j.cbpc.2013.05.002

[75] Lan HCH, Wu KY, Lin IW, Yang ZJ, Chang AA, Hu MCH. Bisphenol a disrupts steroidogenesis and induces a sex hormone imbalance through c-Jun phosphorylation in Leydig cells. Chemosphere. 2017;**185**:237-246. DOI: 10.1016/j.chemosphere.2017.07.004

[76] Ye L, Zhao B, Hu G, Chu Y, Ge RS. Inhibition of human and rat testicular steroidogenic enzyme activities by bisphenol a. Toxicology Letters. 2011;**207**:137-142. DOI: 10.1016/j.toxlet.2011.09.001

[77] Tanaka M, Nakaya S, Katayama M, Leffers H, Nozawa S, Nakazawa R, Iwamoto T, Kobayashi S. Effect of prenatal exposure to bisphenol a on the serum testosterone concentration of rats at birth. Human and Experimental Toxicology. 2006;**25**:369-373. DOI: 10.1191/0960327106ht638oa

[78] Goncalves GD, Semprebon SC, Biazi BI, Mantovani MS, Fernandes GSA. Bisphenol a reduces testosterone production in TM3 Leydig cells independently of its effects on cell death and mitochondrial membrane potential. Reproductive Toxicology. 2018;**76**:26-34. DOI: 10.1016/j.reprotox.2017.12.002

[79] De Freitas AT, Ribeiro MA, Pinho DF, Peixoto AR, Domeniconi RF, Scarano WR. Regulatory and junctional proteins of the blood-testis barrier in human Sertoli cells are modified by monobutyl phthalate (MBP) and bisphenol a (BPA) exposure. Toxicology In Vitro. 2016;**34**:1-7. DOI: 10.1016/j.tiv.2016.02.017

[80] Jin P, Wang X, Chang F, Bai Y, Li Y, Zhou R, Chen L. Low dose bisphenol a impairs spermatogenesis by suppressing reproductive hormone production and promoting germ cell apoptosis in adult rats. Journal of Biomedical Research. 2013;**27**:135-144. DOI: 10.7555/jbr.27.20120076

[81] Singh RP, Shafeeque CM, Sharma SK, Pandey NK, Singh R, Mohan J, Kolluri G, Saxena M, Sharma B, Sastry KVH, Kataria JM, Azeez PA. Bisphenol a reduces fertilizing ability and motility by compromising mitochondrial function of sperm. Environmental Toxicology and Chemistry. 2015;**34**:1617-1622. DOI: 10.1002/etc.2957

[82] Lukacova J, Jambor T, Knazicka Z, Tvrda E, Kolesarova A, Lukac N. Dose- and time-dependent effects of BPA on bovine spermatozoa *in vitro*. Journal of Environmental Science and Health. 2015;**50**:669-676. DOI: 10.1080/10934529.2015.1011963

[83] Rahman MS, Kwon WS, Lee JS, Yoon SJ, Ryu BY, Pang MG. Bisphenol-a affects male fertility via fertility-related proteins in spermatozoa. Scientific Reports. 2015;**5**:9169. DOI: 10.1038/srep09169

[84] Hatef A, Alavi SMH, Linhartova Z, Rodina M, Policar T, Linhart O. *In vitro* effects of BPA on sperm motility characteristics perca fluviatilis L. (Percidae; Teleostei). Journal of Applied Ichthyology. 2010;**26**:696-701. DOI: 10.1111/j.1439-0426.2010.01543.x

[85] Gallart-Ayala H, Moyano E, Galceran MT. Analysis of bisphenols in soft drinks by on-line solid phase extraction fast luquid chromatography-tandem mass spectrometry. Analytica Chimica Acta. 2011;**683**:227-233. DOI: 10.1016/j.aca.2010.10.034

[86] Chen MY, Ike M, Fujita M. Acute toxicity, mutagenicity, and estrogenicity of bisphenol-a and other bisphenols. Environmental Toxicology. 2002;**17**:80-86. DOI: 10.1002/tox.10035

[87] Eladak S, Grisin T, Moison D, Guerquin MJ, N'tumba-Byn T, Pozzi-Gaudin S, Genachi A, Livera G, Rouiller-Fabre V, Habert R. A new chapter in the bisphenol a story: Bisphenol S and bisphenol F are not safety alternatives to this compound. Fertility and Sterility. 2015; **103**. DOI: 11-21. DOI:10.1016/j.fertnstert.2014.11.005

[88] Ji K, Hong S, Kho Y, Choi K. Effects of bisphenol S exposure on endocrine functions and reproduction of zebrafish. Environmental Science and Technology. 2013;**47**:8793-8800. DOI: 10.1021/es400329t

[89] Molina-Molina JM, Amaya E, Grimaldi M, Sáenz JM, Real M, Fernández MF, Balaquer P, Olea N. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-a congeners and derivatives via nuclear receptors. Toxicology and Applied Pharmacology. 2013;**272**:127-136. DOI: 10.1016/j.taap.2013.05.015

[90] Ullah H, Ambreen A, Ahsan N, Jahan S. Bisphenol S induces oxidative stress and DNA damage in rat spermatozoa in vitro and disrupts daily sperm production in vivo. Toxicological and Environmental Chemistry. 2017;**99**:953-965. DOI: 10.1080/02772248.2016.1269333

[91] Ullah H, Jahan S, Ul Ain Q, Shaheen G, Ahsan N. Effect of bisphenol S exposure on male reproductive system of rats: A histological and biochemical study. Chemosphere. 2016; **152**:383-391. DOI: 10.1016/j.chemosphere.2016.02.125

[92] Naderi M, Wong MYL, Gholami F. Developmental exposure of zebrafish (Danio rerio) to bisphenol-S impairs subsequent reproduction potential and hormonal balance in adults. Aquatic Toxicology. 2014;**148**:195-203. DOI: 10.1016/j.aquatox.2014.01.009

[93] Lee S, Liu X, Takeda S, Choi K. Genotoxic potentials and related mechanisms of bisphenol a and other bisphenol compounds: A comparison study employing chicken DT40 cells. Chemosphere. 2013;**93**:434-440. DOI: 10.1016/j.chemosphere.2013.05.029

[94] Fic A, Zegura B, Sollner Dolenc M, Filipic M, Peterlin Masic L. Mutagenicity and DNA damage of bisphenol a and its structural analogues in HepG2 cells. Archives of Industrial Hygiene and Toxicology. 2013;**64**:189-200. DOI: 10.2478/10004-1254-64-2013-2319

[95] Zalmanova T, Hoskova K, Nevoral J, Prokesova S, Zamostna K, Kott T, Petr J. Bisphenol S instead of bisphenol a: A story of reproductive disruption by regretable substitution—A review. Czech Journal of Animal Science. 2016;**61**:433-449. DOI: 10.17221/81/2015-cjas

[96] Kasahara E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, Tokuda M, Nakano Y, Inoue M. Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl) phthalate. Biochemical Journal. 2002;**365**:849-856. DOI: 10.1042/BJ20020254

[97] Albro PW, Jordan ST, Schoeder JL, Corbett JT. Chromatographic separation and quantitative determination of the metabolites of di-(2-ethylhexyl) phthalate from urine of laboratory animals. Journal of Chromatography. 1982;**244**:65-79. DOI: 10.1016/s0021-9673(00)80123-5

[98] Thomas JA, Curto KA, Thomas MJ. MEHP/DEHP: Gonadal toxicity and effects on rodent accessory sex organs. Environmental Health Perspectives. 1982;**45**:85-88. DOI: 10.2307/3429388

[99] Koo JW, Parham F, Kohn MC, Masten SA, Brock JW, Needham LL, Portier CJ. The association between biomarker-based exposure estimates for phthalates and demographic factors

in a human reference population. Environmental Health Perspectives. 2002;**110**:405-410. DOI: 10.1289/ehp.02110405

[100] Akingbemi BT, Youker RT, Sottas CM, Ge R, Katz E, Klinefelter GR, Zirkin BR, Hardy MP. Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl) phthalate. Biology of Reproduction. 2001;**65**:1252-1259. DOI: 10.1095/biolreprod65.4.1252

[101] Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP. Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:775-780. DOI: 10.1073/pnas.0305977101

[102] Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray LEJ. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicological Sciences. 2000; **58**:339-349. DOI: 10.1093/toxsci/58.2.339

[103] Liu K, Lehmann KP, Sar M, Young SS, Gaido KW. Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis. Biology of Reproduction. 2005;**73**:180-192. DOI: 10.1095/biolreprod.104.039404

[104] Clewell RA, Cambell JL, Ross SM, Gaido KW, Clewell HJ, Andersen ME. Assessing the relevance of in vitro measures of phthalate inhibition of steroidogenesis for in vivo response. Toxicology In Vitro. 2010;**24**:327-334. DOI: 10.1016/j.tiv.2009.08.003

[105] Dees JH, Gazouli M, Papadopoulos V. Effect of mono-ethylhexyl phthalate on MA-10 Leydig tumor cells. Reproductive Toxicology. 2001;**15**:171-187. DOI: 10.1016/S0890-6238 (01)00110-1

[106] Jones HB, Garside DA, Liu R, Roberts JC. The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. Experimental and Molecular Pathology. 1993; **58**:179-193. DOI: 10.1006/exmp.1993.1016

[107] Svechnikov K, Svechnikova I, Soder O. Inhibitory effects of mono-ethylhexyl phthalate on steroidogenesis in immature and adult rat Leydig cells in vitro. Reproductive Toxicology. 2008;**25**:485-490. DOI: 10.1016/j.reprotox.2008.05.057

[108] Li X, Fang EF, Scheibye-Knudsen M, Cui H, Qiu L, Li J, He Y, Huang J, Bohr VA, Ng TB, Guo H. Di-(2-ethylhexyl) phthalate inhibits DNA replication leading to hyperPARylation, SIRT1 attenuation, and mitochondrial dysfunction in the testis. Scientific Reports. 2014;**4**. DOI: 10.1038/srep06434

[109] Zhou D, Wang H, Zhang J. Di-n-butyl phthalate (DBP) exposure induces oxidative stress in epididymis of adult rats. Toxicology and Industrial Health. 2010;**27**:65-71. DOI: 10.1177/0748233710381895

[110] Onorato TM, Brown PW, Morris PL. Mono-(2-ethylhexyl) phthalate increases spermatocyte mitochondrial peroxiredoxin 3 and cyclooxygenase 2. Andrology. 2008;**29**:293-303. DOI: 10.2164/jandrol.107.003335

[111] Ichimura T, Kawamura M, Mitani A. Co-localized expression of FasL, Fas, Caspase-3 and apoptotic DNA fragmentation in mouse testis after oral exposure to di(2-ethylhexyl) phthalate. Toxicology. 2003;**194**:35-42. DOI: 10.1016/j.tox.2003.07.003

[112] Richburg JH, Nanez A, Gao H. Participation of the fas-signaling system in the initiation of germ cell apoptosis in young rat testes after exposure to mono-(2-ethylhexyl) phthalate. Toxicology and Applied Pharmacology. 1999;**160**:271-278. DOI: 10.1006/taap.1999.8786

[113] Croxford TP, McCormic NH, Kelleher SL. Moderate zinc deficiency reduces testicular zip6 and zip10 abundance and impairs spermatogenesis. The Journal of Nutrition. 2011; **141**:359-365. DOI: 10.3945/jn.110.131318

[114] Foster PM, Foster JR, Cook MW, Thomas LV, Gangolli SD. Changes in ultrastructure and cytochemical localization of zinc in rat testis following the administration of Di-n-pentyl phthalate. Toxicology and Applied Pharmacology. 1982;**63**:120-132. DOI: 10.1016/0041-008x(82)90031-x

[115] Cater BR, Cook MW, Gangolli SD, Grasso P. Studies on dibutyl phthalate-induced testicular atrophy in the rat: Effect on zinc metabolism. Toxicology and Applied Pharmacology. 1977;**41**:609-618. DOI: 10.1016/s0041-008x(77)80014-8

[116] Cater BR, Cook MW, Gangolli SD. Zinc metabolism and dibutyl phthalate-induced testicular atrophy in the rat. Biochemical Society Transactions. 1976;**4**:652-653. DOI: 10.1042/bst0040652

[117] Lemaire G, Terouanne B, Mauvais P, Michel S, Rahmani R. Effect of organochlorine pesticides on human androgen receptor activation *in vitro*. Toxicology and Applied Pharmacology. 2004;**196**:235-246. DOI: 10.1016/j.taap.2003.12.011

[118] Chatterjee S, Ray A, Bagchi P, Deb C. Suppression of testicular steroidogenesis in rats by the organochlorine insecticide Aldrin. Environmental Pollution. 1988;**51**:87-94. DOI: 10.1016/0269-7491(88)90198-4

[119] Das Neves J, Barnhoorn IEJ, Wagenaar GM. The effects of environmentally relevant concentrations of aldrin and methoxychlor on the testes and sperm of male Clarias gariepinus (Burchell, 1822) after short-term exposure. Fish Physiology and Biochemistry. 2018;**9**:1-14. DOI: 10.1007/s10695-018-0474-4

[120] Mikamo E, Harada S, Nishikawa J, Nishihara T. Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. Toxicology and Applied Pharmacology. 2003;**193**:66-72. DOI: 10.1016/j.taap.2003.08.001

[121] Gizard G, Ouchchane L, Roddier H, Artonne C, Sion B, Vasson MP, Janny L. In vitro alachlor effects on reactive oxygen species generation, motility patterns and apoptosis markers in human spermatozoa. Reproductive Toxicology. 2007;**23**:55-62. DOI: 10.1016/j.reprotox.2006.08.007

[122] Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol a is associated with reduced pituitary

luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology. 2004;**145**:592-603. DOI: 10.1210/en.2003-1174

[123] Ahmed RG. Maternal bisphenol a alters fetal endocrine system: Thyroid adipokine dysfunction. Food and Chemical Toxicology. 2016;**95**:168-174. DOI: 10.1016/j.fct.2016.06.017

[124] Tapiero HT, Nguyen BG, Tew KD. Estrogens and environmental estrogens. Biomedicine and Pharmacotherapy. 2002;**56**:36-44. DOI: 10.1016/S0753-3322(01)00155-X

[125] Tessier D, Matsumura F. Increased ErbB-2 tyrosine kinase activity; MAPK phosphorylation; and cell proliferation in the prostate cancer cell line LNCaP following treatment by select pesticides. Toxicological Sciences. 2001;**60**:38-43. DOI: 10.1093/toxic/60.1.38

[126] Castellanos CG, Sorvik IB, Tanum MB, Verhaegen S, Brandt I, Ropstad E. Differential effects of the persistent DDT metabolite methylsulfonyl-DDE in nonstimulated and LH-stimulated neonatal porcine Leydig cells. Toxicology and Applied Pharmacology. 2013; **267**:247-255. DOI: 10.1016/j.taap.2012.12.022

[127] Diemer T, Allen JA, Hales KH, Hales DB. Reactive oxygen disrupts mitochondria in MA-10 tumor Leydig cells and inhibits steroidogenic acute regulatory (StAR) protein and steroidogenesis. Endocrinology. 2003;**144**:2882-2891. DOI: 10.1210/en.2002-0090

[128] Haavisto TE, Adamsson NA, Myllymäki SA, Toppari J, Paranko J. Effects of 4-tert-octylpheno, 4-tert-butylphenol, and diethlystilbestrol on prenatal testosterone surge in the rat. Reproductive Toxicology. 2003;**17**:593-605. DOI: 10.1016/s0890-6238(03)00103-5