REVIEW PAPER

International Journal of Occupational Medicine and Environmental Health 2018;31(4):377–414
https://doi.org/10.13075/ijomeh.1896.01195

ENVIRONMENTAL EXPOSURE TO NON-PERSISTENT ENDOCRINE DISRUPTING CHEMICALS AND SEMEN QUALITY: AN OVERVIEW OF THE CURRENT EPIDEMIOLOGICAL EVIDENCE

DOROTA ZAMKOWSKA[1], ANETTA KARWACKA[2], JOANNA JUREWICZ[3], and MICHAŁ RADWAN[2]

[1] Medical University of Gdańsk, Gdańsk, Poland
Department of Obstetrics
[2] "Gameta" Hospital, Rzgów, Poland
Department of Gynecology and Reproduction
[3] Nofer Institute of Occupational Medicine, Łódź, Poland
Department of Environmental Epidemiology

Abstract
Some of the recent publications have reported a decline in semen quality in the last few decades. This phenomenon is associated with environmental factors, particularly with exposure to endocrine disrupting chemicals (EDCs). The aim of this publication is to critically review the literature on exposure to the following 6 ubiquitous environmental non-persistent EDCs: bisphenol A, triclosan, parabens, synthetic pyrethroids, organophosphate pesticides and phthalates, and on their influence on semen quality measured as sperm concentration, sperm volume, total sperm count, motility, total motile count, morphology, sperm motion, sperm DNA damage (comet extent, tail length, tail distributed moment, percent of DNA located in the tail (tail%), DNA fragmentation index, high DNA stainability, X:Y ratio and aneuploidy. Several electronic databases were systematically searched until 31 August 2016. Studies were qualified for the review if they: linked environmental exposure to non-persistent EDCs to semen quality outcomes, were published in English after 2006 (and, in the case of phthalates, if they were published after 2009) and were conducted in the case of humans. Out of the 970 references, 45 articles were included in the review. This review adds to the body of evidence that exposure to non-persistent EDCs may affect semen quality parameters and decrease semen quality. Int J Occup Med Environ Health 2018;31(4):377–414

Key words:
Parabens, Semen quality, Environmental exposures, Endocrine disrupting chemicals, Male fertility, Male reproductive system

Received: March 9, 2017. Accepted: August 17, 2017.
Corresponding author: Dorota Zamkowska, Medical University of Gdańsk, Department of Obstetrics, Kliniczna 1a, 80-402 Gdańsk, Poland (e-mail: 33732@gumed.edu.pl).

INTRODUCTION

Over the past few decades endocrine disrupting chemicals (EDCs) have become a significant public health concern. An EDC is defined as "an exogenous chemical or mixture of chemicals, that interferes with any aspect of hormone action" [1]. Humans are at a high risk of exposure to EDCs, as these compounds are ubiquitous in the environment. The uptake of EDCs may occur via various routes: oral route (ingestion of contaminated drinking water and food), dermal contact, inhalation, intravenous route, and transfer from the placenta and maternal milk [2].

The principal mechanism of action of EDCs involves mimicking endogenous hormones and binding to their receptors upon which they may act as agonists or antagonists to alter hormone-regulated cell signaling pathways. Endocrine disrupting chemicals have various hormonal activities, for instance, estrogenic, anti-androgenic, thyroid-disrupting properties. They may also affect various nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs) present in reproductive tissues. Endocrine disrupting chemicals may also directly or indirectly disrupt hormone synthesis and affect steroidogenesis [3]. Through these and many other pathways, which have been modeled in both *in vitro* and *in vivo* studies, EDCs may affect the male and the female reproductive systems, the mammary gland development and breast cancer, prostate cancer, reproductive neuroendocrine systems, the thyroid, metabolism and obesity, and cardiovascular endocrinology [4].

Studies of environmental EDCs have suggested that persistent organic pollutants (POPs), such as: dioxins, polychlorinated biphenyls (PCBs) and some pesticides resist environmental degradation and are slowly metabolized in the body. These chemicals accumulate in lipophilic tissues, biomagnify through the food chain and have been found globally, even in regions where they have never been used [5]. On the other hand, environmental chemicals, such as: bisphenol A, phthalates, parabens and synthetic pyrethroids are classified as non-persistent because they readily decompose in the environment, are rapidly metabolized in the body, have also been reported to have endocrine disrupting properties and are suspected to affect human reproduction and development [4].

Infertility has become a major problem of our times. It is estimated that as many as 15% of couples fail to conceive after a year of attempts. It is believed that in 20% of infertile couples, the main cause of infertility is the male factor and that it is a contributing factor of infertility in a further 30–40% of infertile couples [6]. The interest in the impact of exposure to EDCs, especially non-persistent chemicals, on the male and female reproductive systems has been increasing [7]. The association between EDCs and male infertility has been investigated in numerous animal and human studies [3]. One of the examined issues has been the impact of EDCs on semen quality, as a decreasing sperm quality has been reported in the past few decades [8].

The aim of this paper is to critically review the literature on the exposure to the following environmental non-persistent chemicals: bisphenol A, triclosan, parabens, synthetic pyrethroids, organophosphate pesticides and phthalates, and on their effects on semen quality measured as: sperm concentration, sperm volume, total sperm count, motility, total motile count, morphology, sperm motion, sperm DNA damage (comet extent, tail length, tail distributed moment (TDM), percent of DNA located in the tail (tail%), DNA fragmentation index (DFI), high DNA stainability (HDS), X:Y ratio, and aneuploidy.

MATERIAL AND METHODS

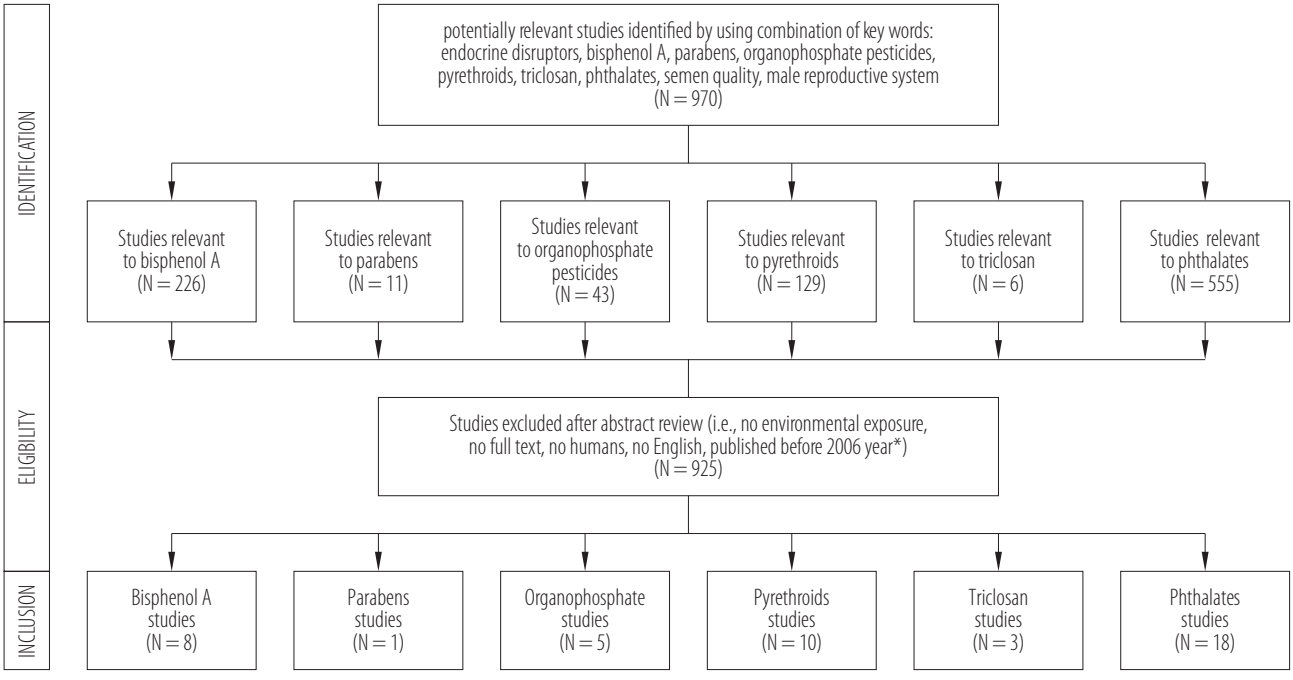
Epidemiological studies focusing on the exposure to non-persistent environmental chemicals and semen quality were identified by a search of multiple literature databases (i.e., MEDLINE, PubMed, Web of Science, EBSCO, Scopus) (before December 2016). The search combined terms referring to the exposure to environmental EDCs

and semen quality. The combination of the key words used were:

– those referring to the exposure: environmental exposure to non-persistent endocrine disrupting chemicals, exposure to phthalates, bisphenol A, triclosan, parabens, organophosphate pesticides, and to synthetic pyrethroids;
– those referring to the outcome: semen quality measured as sperm concentration, sperm volume, total sperm count, motility, total motile count, morphology, sperm motion, sperm DNA damage (comet extent, tail length, TDM, tail%, DFI, HDS), X:Y ratio, and aneuploidy.

Relevant studies were also identified through a review of the references cited in all the published studies. Only original articles were included. We excluded studies that analyzed the impact of environmental EDCs on pregnancy as well as those assessing the effects of lifestyle factors (smoking, alcohol consumption, diet) and occupational exposure studies. Articles focused on animal research, *in vitro* studies and review papers were excluded. Finally, this review included human studies published in English in peer-reviewed journals since 2006. This period was chosen because there were few studies conducted on semen quality and exposure to non-persistent environmental EDCs prior to 2006. At that time, the availability of sensitive, specific, and affordable bioassays made biomarkers feasible for use in epidemiological studies for measuring exposures to those compounds. At the same time, growing rodent literature provided convincing data on the reproductive toxicity of several non-persistent environmental EDCs.

All the full-text articles were thoroughly examined to identify the aims of the studies, statistical methods and accurate results. All the related data was extracted independently by 2 investigators and incongruences were resolved by discussion and intervention of a third independent author. In summary, out of the 970 articles identified, 45 met the eligibility criteria and have been included in this review (Figure 1).



* Phthalates studies excluded if published before 2009 year.

Fig. 1. Flow chart of study selection for systematic review of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters

RESULTS

Exposure to bisphenol A and semen quality

The estrogenic properties of bisphenol A (BPA) have been known since the 1930s [9]. Bisphenol A has also been known for its anti-androgenic and anti-thyroid activities. Like other EDCs, it may affect nuclear receptors and interfere with their signaling pathways but it may also act through non-genomic pathways initiated at membrane receptors. It acts as an agonist of the estrogen receptor alfa and as an antagonist of the androgen, thyroid and aryl hydrocarbon receptors [10,11]. Bisphenol A is widespread in the environment and it is used in the manufacture of polycarbonate plastic, epoxy resins, multiple domestic products and medical devices. Humans exposure to BPA occurs through the diet, air, dust and water. It may migrate from containers into liquids at high temperatures [12]. Most samples of body fluids and tissues collected from humans have been found to contain quantifiable levels of BPA [13].

Male reproductive function may be affected by exposure to BPA *in utero*, during puberty and adulthood [11]. Animal studies investigating the intrauterine exposure to BPA have shown that male reproductive function may be impaired in multiple ways. Bisphenol A has been shown to affect the structure of the testes, prostate and epididymides, to influence the anogenital distance (AGD), to reduce the expression of hormones and to alter the gene expression profile. It may also impair the development of hypothalamus and affect the expression of thyroid-specific genes [11]. *In vivo* studies have reported similar findings.

Postnatal exposure to BPA may impair spermatogenesis, sperm function and sperm quality as a result of effects on the hypothalamic-pituitary-testicular axis [11]. Testicular function may also be impaired by pro-oxidant/antioxidant imbalance of testicular cells, by decreased activities of antioxidant enzymes, and by lipid peroxidation induced in epididymal and sperm cells [11]. Some evidence also suggests that BPA may directly affect spermatozoa through its action on fertility-related proteins present in these cells [14,15]. Lower semen quality after exposure to BPA has been observed in rodent studies [16,17]. Epidemiological studies in humans seem to confirm that even environmental exposure to BPA may impair semen quality.

The association between environmental exposure to BPA and semen quality were examined in 8 studies [18–25]. Four of these studies had been conducted among men representing the general population [18,21,22,25] and the subsequent 4 – among men managed at fertility clinics [19,20,23,24]. Most of these studies assessed exposure to BPA by measuring total urinary concentrations of BPA [18–22,25], while only 2 studies assessed unconjugated BPA in plasma and seminal plasma [23,24].

In the first prospective cohort study of fertile young men from 4 cities in the United States, Mendiola et al. (2010) found no significant association between any examined semen parameters (seminal volume, concentration, motility, morphology, total motile count, total sperm count) and urinary BPA concentration [18]. On the other hand, Lassen et al. (2014), who also examined the quality of semen sampled from healthy young men attending a compulsory physical examination for military service, reported a significant inverse association between BPA concentration in urine and progressive sperm motility [21]. A study conducted between 2005 and 2009 investigated semen parameters in the general population represented by men from Michigan and Texas, USA. The authors of this study found a negative relationship between BPA and DNA fragmentation, suggesting less sperm DNA damage [22]. Li et al. (2011) examined workers with environmental exposure to BPA and observed an inverse association between urine BPA and both sperm concentration and total sperm count [25].

Results of the studies performed among men managed at fertility clinics showed a tighter interrelationship between BPA concentration and semen quality. Meeker et al. (2010), recruited partners in subfertile couples

seeking treatment from a fertility clinic in Massachusetts, USA, and found that the increase in the interquartile range (IQR) of urinary BPA was associated with 23% decline in sperm concentration, 7.5% decline in motility and 13% decline in morphology along with 10% increase in sperm DNA damage measured as the percentage share of DNA in comet tail [19]. These findings were consistent with the results of a study performed in Slovenia among men who were also recruited through a fertility clinic. This study found inverse relationships between total urinary BPA concentration and the following: sperm concentration, total sperm count and total motile sperm [20]. The authors of 2 recent studies, in which BPA was measured in human plasma and seminal fluid, found an inverse association of seminal BPA with sperm concentration and total sperm count [23,24].

In conclusion, the diverse outcomes may be due to the differences in the selection of study groups and biological fluids in which concentrations of BPA were measured. However, most of these human studies showed a significant negative association between BPA concentration in biological matrices and semen quality [19–21,23–25].

Exposure to triclosan and semen quality

Triclosan (TCS) is suspected to pose a risk to developmental and reproductive human health [1]. While non-human studies have shown sufficient evidence of its possible toxic activity, there is not enough evidence from human studies, due to their small number, to associate TCS with negative effects on developmental and reproductive human health [26]. The number of human studies is, however, growing rapidly, as TCS has become a cause of concern due to its ubiquity in the environment. The manufacture of TCS on a massive scale started in the 1970s, and 20 years later the compound reached the top 10 detected contaminants in American rivers [27]. Because of its antibacterial properties TCS is used in personal care products and as an ingredient of soups, cosmetics and toothpastes. It may be also found in toys, kitchenware, clothes and furniture [28]. From domestic wastewater, TCS migrates to wastewater treatment plants where it is absorbed into the settled sewage sludge, which may, in turn, be transformed into biosolids and used as agricultural fertilizers [29]. Food and water may be another exposure source of TCS for humans.

The main routes of absorption are through the skin, mucous membranes and gastrointestinal tract. After absorption in humans, TCS may be detected in urine, blood, milk, plasma, brain, adipose tissue and liver [30]. Animal studies have found evidence linking TCS exposure to reproductive and developmental health [26]. The mechanism of action of TCS is unclear. *In vitro* studies have demonstrated that TCS may bind with low affinity with estrogen and androgen receptors to act as their agonist, antagonist or to result in no action [31]. It adversely affects the male reproductive system by disrupting steroidogenesis. Kumar et al. (2008) conducted an *in vitro* study in rodent Leydig cells and found that TCS depressed the synthesis of cyclic adenosine monophosphate (cAMP) resulting in the disruption of the steroidogenic cascade and leading to decreased testosterone synthesis [32]. Forgacs et al. (2012) found that high doses (30 μM) of TCS inhibited testosterone synthesis but only recombinant human chorionic gonadotropin (rhCG) induced synthesis, while basal testosterone production remained unaffected [33].

Kumar et al. (2009) carried out an *in vivo* study in rodent Leydig cells and found that higher doses of TCS (10 mg/kg/day and 20 mg/kg/day) caused a significant decrease in testis weight and sex accessory tissues. Another finding was the downregulation of testicular levels of mRNA for cytochrome P450scc, cytochrome P450c17, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), testicular steroidogenic acute regulatory protein (StAR), androgen receptor (AR) and a decreased *in vitro* activity of testicular steroidogenic enzymes. They also reported decreased levels of serum luteinizing hormone (LH), follicle stimu-

lating hormone (FSH), cholesterol, pregnenolone and testosterone. All these findings were followed by decreased semen production [34]. Two other *in vivo* studies in the same animal species did not, however, corroborate these results [35,36].

Studies in humans are lacking. Only 3 studies were identified assessing the relationship between the exposure to TCS and semen quality [37–39]. Zhu et al. (2016) measured urinary TCS concentration in men recruited through reproductive health clinics and found an inverse association between urine TCS overall concentration and the number of forward moving sperms. They also found that TCS was negatively associated with sperm concentration, sperm count, the number of forward moving sperms, and the percentage share and the number of morphologically normal sperms but only in the lowest tertile of urinary TCS concentration (< 0.66 ng/mg). There was no significant association between urinary TCS and semen quality in the tertiles of middle and high urinary TCS levels [37].

The second of these 3 studies investigated the association of the exposure to TCS with idiopathic male infertility and found no relationship [38]. The findings reported in this study are consistent with those reported in a study performed in Belgium. The authors of the latter investigated whether exposure to TCS and other EDCs was associated with increased subfertility in men. In this study, no relationship between the exposure to TCS and sperm quality parameters was found, either [39].

The divergence of the results in these 3 studies may be due to the use of various methods of urinary TCS quantification and diverse statistical models. In light of the small number of studies and the divergent results, further studies are required.

Exposure to parabens and semen quality

Parabens are the family of para-hydroxybenzoic acid esters which are used as preservatives in cosmetic products, pharmaceuticals and food. Dermally applied cosmetics are the primary sources of exposure. Uptake into the skin increases with lipid solubility, which in turn depends on the length of the ester chain. Oral intake is another route of exposure [40]. After absorption, parabens do not accumulate in the body but are metabolized by esterases and conjugated, and excreted with urine, bile and faeces [41]. The most commonly used parabens are methylparaben (MP), propylparaben (PP) and butylparaben (BP). Because of their relatively low toxicity, parabens have become the most widely used preservatives since their first synthesis in the 1930s [42].

Parabens attracted public attention after a publication in 2004 which reported that parabens had been identified in human breast tumor tissue [43]. Following the growing anxiety, the European Union adopted a regulation in 2015 which decreased the maximum concentrations of PP and BP to 0.14% in mixed and individual forms and banned these compounds from leave-on products for the nappy area of children below 3 years of age. The main concern relates to their possible endocrine disrupting activity. Parabens are primarily associated with the development of breast cancer, allergic contact dermatitis and skin inflammation [44], and with the disruption of the reproductive system [41]. Estrogenic and anti-androgenic properties of parabens have been confirmed in many *in vitro* and *in vivo* studies [41]. However, studies confirming potential harmful effects of parabens on animals are lacking.

In a recently published study assessing the disrupting effects in rats prenatally exposed to butylparaben, multiple adverse effects on the reproductive system (shortened AGD, reduced reproductive organ weight, disrupted testicular gene expression, inappropriate mammary gland development and significantly reduced sperm count) were found. The authors emphasized that they had observed lower sperm count even after exposing rats to small doses of 10 mg/kg [45]. The influence of butylparaben after intrauterine exposure on sperm count was also reported in other studies in rats, although the exposure levels were

much higher [46,47]. Several mechanisms are suspected to be responsible for the disruption of the reproductive system. Chen et al. reported that MP, PP and BP were anti-androgens and might inhibit testosterone-dependent transcription by 40%, 33% and 19%, respectively [48].

Another mechanism which has been investigated is the disruption of testicular gene expression. After oral exposure of Wistar rats to butylparaben from gestation day 7 to pup day 22, an increased Cyp19a1 (aromatase) expression in testes was observed in all the exposure groups as compared with controls. Aromatase was reduced in prepubertal but not in the case of adult rats exposed to butylparaben. The authors speculated that the reduction of aromatase levels in this period was associated with low sperm count observed later in life. Another finding in this study was the reduced expression of Nr5a1, a gene encoding the nuclear receptor steroidogenic factor-1 (SF-1), which regulated multiple genes that might indicate persistent disruption of steroidogenesis [45]. Tavares et al. have suggested that disruption of sperm function may be caused by interference of parabens with mitochondrial energetics [49].

Studies associating human exposure to parabens and semen quality are lacking. Meeker et al. (2010) investigated urinary concentration of parabens and its association with sperm quality parameters and sperm DNA damage [50]. The study was conducted in the same population of male partners in subfertile couples recruited through a fertility clinic that was described in a study of this author linking exposure to BPA to semen quality [19]. Concentrations of total urinary parabens (MP, PP, BP) were measured. No statistically significant association between MP and PP on the one hand and semen quality and sperm DNA damage on the other was found. Urinary BP concentrations were not found to be associated with semen quality parameters. However, a dose-related positive association between BP urine concentration and increased tail% was found [50].

The lack of comparative studies restricts our ability to make any conclusions. Further studies are necessary.

Exposure to synthetic pyrethroids and semen quality

Synthetic pyrethroids are analogues and derivatives of the original pyrethrins naturally found in flowers of the chrysanthemum family. They are used as pesticides in households, in agriculture and in veterinary practices. This group of pesticides is widely used because of its effectiveness in contact with insects, low mammalian toxicity, and biodegradability. Pyrethroids act by modifying sodium and chloride channels in the axons to alter the normal function of nerves [51,52]. The main route of exposure, apart from occupational exposure, is the diet: consumption of raw and cooked vegetables and fruits has been associated with increased detection of pyrethroid metabolites [53]. Exposure may also occur by inhalation of contaminated household dust and by dermal contact [51].

After absorption pyrethroids are rapidly metabolized and excreted with urine. The most frequently detected metabolite in human urine is 3-phenoxybenzoic acid (3PBA), which is a metabolite of the 6 pyrethroid pesticides (tralomethrin, fenpropathrin, cypermethrin, deltamethrin, permethrin, cyhalothrin). The other metabolites are *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (TDCCA) and *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (CDCCA), which are metabolites of permethrin, cypermethrin, cyfluthrin; *cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA), which is a metabolite of deltamethrin; and 4-fluoro-3-phenoxybenzoic acid (4F3PBA), a metabolite of cyfluthrin. Urinary levels of these metabolites may reflect multiple routes of environmental exposure to their parent pyrethroid pesticides and their environmental degradants [54].

Epidemiological and animal studies have shown a negative impact of pyrethroids on reproductive health [55]. Pyrethroids have been identified as potential endocrine disruptors [56]. They may act as agonists of the estrogen receptors and as antagonists of the androgen receptors, and show anti-androgenic properties [57,58]. *In vitro*

and *in vivo* studies have shown that pyrethroids may induce genotoxicity and oxidative stress by promoting the formation of reactive oxygen species [59–61]. Animal studies have reported that prenatal exposure to pyrethroids may impair development of the testes and epididymides, decrease the number of epididymal spermatozoa in adult male offspring, disrupt spermatogenesis, and decrease testosterone synthesis by downregulating the expression of testicular StAR [62,63]. These studies have also shown a negative influence of pyrethroids on semen quality [64,65]. The negative impact of synthetic pyrethroids on the male reproductive system is increasingly investigated in humans. Most of the studies so far have reported a negative impact of pyrethroids on hormone synthesis [66], sperm quality and DNA damage [67–69].

This review identified 10 studies conducted after 2006, that assessed environmental exposure to pyrethroids and semen quality. Most of the studies were performed among men from fertility clinics [67–72] and only 2 studies were conducted among men from the general population [73,74].

The studies conducted among men from fertility clinics were consistent in their findings: they all showed associations between some of the examined semen parameters (concentration [68–70], motility [68,71], sperm motion [68,70], sperm DNA damage [67–69], sperm sex ratio [72], sperm aneuploidy [75,76] and exposure to pyrethroids. Xia et al. (2008) found an association between increased concentration of urinary 3-PBA levels and sperm concentration and sperm motion parameters [71]. Meeker et al. (2008) found a positive association between 3PBA and sperm concentration and sperm DNA damage.

Additionally, an inverse association between TDCCA and sperm motility and sperm motion was found [68]. In the study conducted in China in 2011, the authors found a strong relationship between urinary 3-PBA levels and sperm concentration and sperm DNA fragmentation [69]. In a small pilot study conducted by Toshima et al. (2012), a significant inverse association between urinary 3BPA concentration and sperm motility was also found [70]. Jurewicz et al. (2015) investigated the relationship between environmental exposure to pyrethroids and sperm DNA damage and found a positive association between CDCCA concentration > 50th percentile and the percentage share of medium DNA fragmentation index (M DFI) and the percentage share of high DNA stainability (HDS). They also found an association between urinary 3PBA concentration > 50th percentile and the percentage share of high DNA fragmentation index (H DFI) [67].

Studies in which the association between aneuploidy rates and the exposure to pyrethroids was measured were also consistent. Young et al. (2013) found that urinary concentrations of CDCCA and TDCCA above the limit of detection (LOD) were associated with increased rates of aneuploidy [75]. In a similar study, the authors reported that urinary concentrations of CDCCA, TDCCA and 3PBA in men affected sperm chromosome disomy of chromosome 18 (CDCCA, 3PBA), XY (TDCCA, 3PBA), YY (3PBA), 21 (3PBA) and total disomy (3PBA) [76]. Additionally, one study investigated the association between urinary concentration of synthetic pyrethroids and sperm Y:X ratio. The authors reported negative associations of the concentration of CDCCA to TDCCA with Y:X sperm chromosome ratio [72].

As already mentioned, this review identified only 2 studies performed among men from the general population. Perry et al. (2007) conducted a small pilot study in which they reported that sperm concentration was lower in the group of higher environmental exposure [73]. In contrast to these findings, the authors of another study performed among healthy young students did not find any evidence of adverse influence of environmental exposure to synthetic pyrethroids on semen quality [74].

Most of the studies showed that exposure to synthetic pyrethroids was associated with a negative impact on semen quality and only one of the identified studies showed inconsistent results.

Exposure to organophosphate pesticides and semen quality

Organophosphate pesticides (OPPs) are esters of phosphoric acid. They are used as insecticides and herbicides in agriculture, households and veterinary practices [77]. The organophosphate compounds tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) are also used as flame retardants in polyurethane foams and plasticizers [78]. Because of their effectiveness and non-persistence in the environment they have replaced other pesticides, such as organochlorines, and have become a large percentage share of all insecticides used worldwide. They act by inhibiting cholinesterases, particularly acetylcholinesterase, an enzyme found in the nervous system, neuromuscular junctions and erythrocytes. Inhibition of this enzyme results in the accumulation of acetylcholine at the synapses and causes overstimulation of acetylcholine receptors [79].

The general population is exposed mainly through ingestion of contaminated food and contact with surfaces containing organophosphorus insecticides, with less common routes being inhalation and dermal contact [80]. After absorption, OPPs are rapidly metabolized. About 75% of the approved OPPs are metabolized to at least one of the 6 commonly measured dialkyl phosphates (DAPs), namely dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP). Urinary DAP metabolites are not considered toxic. Concentrations of these metabolites are used as a biomarker of recent exposure to OPPs or to the metabolite itself [81].

Studies in animals and occupationally exposed humans have shown that pesticides may cause multiple histopathological and cytopathological changes in the male reproductive system [82]. They may cause significant testicular damage [83], adversely affect the structure and function of the male accessory reproductive organs [84], alter reproductive hormone levels [85], and decrease spermatogenesis [86,87] and semen quality [88]. The mechanism of the disruptive effects of OPPs on male reproductive function is unclear. These compounds have been investigated for their endocrine disrupting properties and genotoxicity [56,85,89,90]. Organophosphate pesticides are structurally similar to sex steroid hormones and due to this similarity they may bind to endocrine receptors, act as hormonal ligands, and disrupt gene transcription [91]. They are known to affect the hypothalamic-hypophyseal-gonadal (HHG) system at multiple levels by mimicking sex steroid hormones [92]. They may also increase apoptosis of germ cells by disturbing tissue homoeostasis. Like other EDCs, they induce oxidative stress and cause genotoxicity [91].

To date, few studies have evaluated the impact of environmental exposure to OPPs on semen quality. Two studies have been performed among men from the general population [73,93] and 3 among men from fertility clinics [94–96]. In most of the studies, the authors measured OPP exposure by measuring urinary concentration of organophosphate metabolites [73,93,95,96], and in one of the study, the authors assessed the relationship between TDCPP and TPP concentrations in house dust and semen quality parameters [94].

In a pilot study assessing the exposure to OPPs, Perry et al. (2007) found a statistically significant association between DETP and sperm concentration [73]. This relationship was investigated further in a larger study where a significant association between DMP and sperm concentration and motility was found [93]. In another study conducted among men from fertility clinics who had been exposed to organophosphates in their households, the authors found evidence that concentrations of OPPs in house dust may be associated with decreased sperm concentration [94]. The results of a study conducted by Melgarejo et al. also suggest that exposure to OPPs may be associated with decreased sperm counts and motility [95].

The authors of a more recent study, in which environmental exposures to OPPs and their association with the frequency of sperm chromosomal abnormalities were investigated, reported that urinary DAP metabolites were associated with increased disomy rates [96].

Because of the limited number of studies investigating the impact of environmental OPP exposure on human health further characterization of the issue in epidemiological studies is needed.

However, the results of the studies presented here were consistent with each other and suggest that environmental exposure to OPPs may negatively affect sperm quality.

Exposure to phthalates and semen quality

Phthalates are chemicals used as plasticizers in hundreds of products, such as personal care products, medical devices, food packaging, and toys [97]. The industrial applications of phthalates are related to the length of the ester chain. They are divided into 2 distinct groups, with very different applications, toxicological properties and classification. High-molecular-weight phthalates (e.g., di(2-ethylhexyl) phthalate (DEHP)), with alkyl chain lengths from 8 to 13 carbons, are widely used as general-purpose plasticizers in polymers, primarily in polyvinyl chloride (PVC) resins [98] to make rigid PVC more flexible and useful, such as for wiring and cables. These phthalates are also used in a variety of consumer products, flooring and wall coverings, in food contact application and medical devices (bags for blood and parenteral nutrition, tubings and catheters) [97]. Low-molecular-weight phthalates, with alkyl chain lengths from 2 to 7 carbons (e.g., diethyl phthalate (DEP), dibutyl phthalate (DBP)) are used in personal care products, some cosmetics/fragrances, lacquers and varnishes, and as solvents and plasticizers in cellulose acetate [98].

Human exposure to phthalates occurs mainly through ingestion, dermal exposure and inhalation. After absorption, phthalates are rapidly metabolized to monoesters or oxidative metabolites, and excreted free or conjugated as glucuronides in the urine and faeces. Urinary concentrations of phthalate metabolites have been used as the most common biomarker of human exposure [98]. Because of their ubiquity they began to be investigated for potential consequences for human health. Their endocrine disrupting properties are well known. Phthalates mainly act as anti-androgens, although they may also manifest weak estrogenic properties [99]. Some of them have been found to cause reproductive toxicity in animals [100]. They have been linked to hypospadias or cryptorchidism, timing of puberty onset, AGD, reproductive hormones and semen quality.

However, the evidence in animal and epidemiological studies for most of their adverse effects in the reproductive system is still insufficient, and the evidence of their disrupting effect on semen quality is only moderately strong [101]. The potential mechanism of adverse effects of phthalates on semen quality involves causing morphological alterations of the testis including a decrease in Sertoli cells, disruption of the seminiferous tubules and formation of multinucleated germ cells. They may also induce Leydig cell dysfunction that leads to the inhibition of steroidogenic enzymes. Furthermore, they have been found to disrupt the patterns of gene expression which are important to cholesterol transport and steroidogenesis [101].

Multiple epidemiological human studies have assessed the relationship between the exposure to phthalates and semen quality. Due to the fact that the authors of this study have already reviewed studies linking the exposure to phthalates to male reproductive outcomes (including semen quality) which were published until 2009 year [102], this review has been narrowed down to the papers published after the year 2009, identifying a total of 18 such studies in which the exposure to phthalates and their influence on semen parameters, DNA damage, X:Y ratio and sperm aneuploidy were assessed. Most of these studies were conducted among men recruited by fertility clinics (Table 1).

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
Bisphenol A (BPA)					
Mendiola et al. (2010) [18], United States	prospective cohort study	geometric mean: BPA = 1.5 μg/l	total urinary concentration of BPA (free + conjugated species) BPA LOD = 0.4 mcg/l single urine sample collected	375 fertile men from 4 U.S. study centers (Missouri, Iowa, Minnesota, California)	no significant associations between any semen parameters and urinary BPA concentration: seminal volume (β = –0.18, 95% CI: –0.4–0.01) sperm concentration (β = 0.01, 95% CI: –0.08–0.1) motile sperm (%) (β = –0.38, 95% CI: –1.66–0.9) morphologically normal sperm (%) (β = 0.16, 95% CI: –0.45–0.77) total motile count (β = –0.05, 95% CI: –0.17–0.7) total sperm count (β = –0.04, 95% CI: –0.14–0.06)
Meeker et al. (2010) [19], United States	cross-sectional	geometric mean: uncorrected BPA = 1.4 ng/ml SG-corrected BPA = 1.7 ng/ml	total urinary concentration of BPA (free + conjugated species) BPA LOD = 0.4 mcg/l 3 urine samples collected	190 men recruited through U.S. infertility clinic	IQR increase in urinary BPA was associated with declines in sperm concentration 23% (95% CI: –40–(–0.3)%), motility 7.5% (95% CI: –17–1.5%), and morphology 13% (95% CI: –26–(–0.1)%), along with a 10% (95% CI: 0.03–19%) increase in sperm DNA damage measured as tail%
Li et al. (2011) [25], China	cohort study	median CR-adjusted: BPA = 1.4 μg/g CR	total urinary concentration of BPA (free + conjugated species) LOD BPA = 0.31 mcg/l single urine sample collected	88 Chinese workers exposed only to environmental BPA level	inverse association between urine BPA and sperm concentration (p = 0.02) and total sperm count (p = 0.04)
Knez et al. (2014) [20], Slovenia	prospective cohort study	geometric mean: BPA = 1.55 ng/ml	total urinary concentration of BPA (free + conjugated species) LOD BPA = 0.1 ng/ml single urine sample collected	149 men recruited through Slovenia infertility clinic	inverse relationship between total urinary BPA concentrations and sperm concentration (p = 0.047), total sperm count (p = 0.039), sperm vitality (p = 0.026) and total motile sperm (p = 0.043)

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
Lassen et al. (2014) [21], Denmark	cross-sectional	median: BPA = 3.25 ng/ml	total urinary concentration of BPA (free + conjugated species) LOD BPA = 0.12 ng/ml single urine sample collected	298 young Danish men from the general population	no association between semen volume ($p = 0.95$), sperm concentration ($p = 0.56$), total sperm count ($p = 0.71$) or percentage morphologically normal forms ($p = 0.79$) significant inverse association between BPA and progressive motility ($p = 0.003$)
Goldstone et al. (2015) [22], United States	prospective cohort study	geometric mean: BPA = 1.62 ng/ml	total urinary concentration of BPA (free + conjugated species) LOD BPA = 0.05 ng/ml single urine sample collected	418 men from 16 counties in Michigan and Texas from the general population	negative relation between BPA and DNA fragmentation – less sperm DNA damage ($\beta = -0.0544$, $p = 0.035$)
Vitku et al. (2015) [23], Czech Republic	cross-sectional	mean plasma BPA: group 1 – 47 pg/ml group 2 – 137 pg/ml group 3 – 114 pg/ml group 4 – 33 pg/ml mean seminal plasma BPA: group 1 – 66 pg/ml group 2 – 144 pg/ml group 3 – 132 pg/ml group 4 – 179 pg/ml	unconjugated BPA in plasma and seminal plasma single plasma and seminal plasma sample collected	174 Czech men with diverse degrees of infertility[a]: group 1 – N = 84 group 2 – N = 56 group 3 – N = 20 group 4 – N = 14	inverse association between seminal BPA and sperm concentration ($p < 0.001$) and total sperm count ($p < 0.01$)
Vitku et al. (2016) [24], Czech Republic	cross-sectional	mean plasma BPA: group 1 – 0.029 ng/ml group 2 – 0.059 ng/ml group 3 – 0.072 ng/ml group 4 – 0.019 ng/ml mean seminal plasma BPA: group 1 – 0.075 ng/ml group 2 – 0.130 ng/ml group 3 – 0.153 ng/ml group 4 – 0.148 ng/ml	unconjugated BPA in plasma and seminal plasma single plasma and seminal plasma sample collected	191 Czech men with diverse degrees of infertility[a]: group 1 – N = 89 group 2 – N = 59 group 3 – N = 25 group 4 – N = 18	inverse association between seminal BPA and sperm concentration ($p = 0.009$), total sperm count ($p = 0.018$) and morphology ($p = 0.044$)
Triclosan (TCS)					

Chen et al. (2013) [38], China	cross-sectional	geometric mean of TCS: controls – 1.593 ng/ml cases – 1.707 ng/ml	total urinary TCS (free and conjugated) LOD = 0.34 ng×ml^{-1} single urine sample collected	1 590 men recruited through hospitals affiliated to Nanjing Medical University case group – 877 idiopathic infertile men	no significant associations between any semen parameters and urinary triclosan concentration
Den Hond et al. (2015) [39], Belgium	cross-sectional	geometric mean of unadjusted TCS: controls – 2.8 µg/l cases – 2.6 µg/l	total urinary TCS (free and conjugated) single urine sample collected	120 healthy men recruited through fertility clinic	no relationships between exposure to TCS and sperm quality parameters sperm concentration ($p = 0.33$), motility ($p = 0.71$), morphology ($p = 0.99$)
Zhu et al. (2016) [37], China	cross-sectional	geometric mean: TCS = 1.12 ng/ml CR-corrected TCS = 0.99 ng/mg	total urinary TCS (free and conjugated) LOD = 0.1 µg/l single urine sample collected	471 men recruited through reproductive health clinic	inverse association between overall triclosan concentration and number of forward moving sperms ($\beta = -0.17$, 95% CI: –0.32–(–0.02)) in tertile of triclosan level < 0.66 ng/mg negative association between triclosan and sperm concentration ($\beta = -0.21$, 95% CI: –0.41–(–0.01)), total sperm count ($\beta = -0.25$, 95% CI: –0.48–(–0.02)), number of forward moving sperms ($\beta = -0.35$, 95% CI: –0.68 –(–0.03)), percentage of normal morphologic sperms ($\beta = -1.64$, 95% CI: –3.05–(–0.23)) and number of normal morphologic sperms ($\beta = -0.48$, 95% CI: –0.8–(–0.16))
Parabens					
Meeker et al. (2010) [50], United States	cross-sectional	geometric mean: MP = 28.6 µg/l PP = 3.67 µg/l BP – not calculated SG-corrected: MP = 35.5 PP = 4.52 BP – not calculated	total urinary concentration of BP, MP, PP (free and conjugated species), MP LOD = 1 µg/l PP and BP LOD = 0.2 µg/l 3 urine sample collected	190 male partners recruited through U.S. infertility clinic	no statistically significant association between urinary concentration of parabens and semen quality parameters (N = 190) (all p for trends > 0.05): urinary BP levels were positively associated with sperm DNA damage (N = 137) increase in tail% (p for trend = 0.03)

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
Pesticides					
Perry et al. (2007) [70], China	cross-sectional pilot study	geometric mean: DMTP = 5.7 µg/l DETP = 4.27 µg/l PNP = 6.9 µg/l	urinary concentration of DMTP, DETP, PNP DMTP, DETP LOD = 0.25 µg/l PNP LOD = 0.14 µg/l single urine sample collected	18 men with environmental exposure	statistically significant association between DETP and sperm concentration Log (sperm concentration) difference = –1.0 (95% CI: –1.8–(−0.2))
Meeker et al. (2010) [94], United States	cross-sectional	mean: TDCPP = 1.88 ng/g dust TPP = 7.4 ng/g dust	house dust concentrations of TDCPP and TPP TDCPP LOD = 107 ng/g TPP LOD = 173 ng/g single sample of house dust collected	50 men recruited through U.S. infertility clinic	association between IQR increase in TPP and decreased sperm concentration 18.8% (95% CI: –30.1–(–4.5)%) (p = 0.02 after excluding 3 men with sperm concentration < 20 sperm/ml)
Perry et al. (2011) [93], China	cohort study	mean: DEDTP: controls – 0.15 µg/l, cases – 0.14 µg/l DMP: controls – 2.93 µg/l, cases – 3.96 µg/l DMTP: controls – 4.14 µg/l, cases – 3.36 µg/l DEP: controls – 8.39 µg/l, cases – 6.90 µg/l DETP: controls – 23.00 µg/l, cases – 18.00 µg/l DMDTP: controls – 0.23 µg/l, cases – 0.24 µg/l	urinary concentration of DMP, DMTP, DMDTP, DEP, DETP, DEDTP DEDTP, DMDTP, DEP, DETP LOD = 0.125 µg/l DMP, DMTP LOD = 0.25 µg/l single urine sample collected	189 male partners of recently married couples	significant association between DMP and semen quality (OR = 1.3, 95% CI: 1.02–1.65)
Melgarejo et al. (2015) [95], Spain	cross-sectional	geometric mean: DMP = 1.3 µg/l DMTP = 1 µg/l DMDTP = 0.08 µg/l DEP = 2.6 µg/l DETP = 0.94 µg/l DEDTP = 0.05 µg/l	urinary concentration of DMP, DMTP, DMDTP, DEP, DETP, DEDTP; DEDTP LOD = 0.01 mg/l DMP, DMTP, DMDTP, DEP, DETP LOD = 0.1 µg/l single urine sample collected	116 men recruited through infertility clinic	significant inverse correlation between concentration of DMP and the % of motile sperm (r = –0.23, 95% CI: –0.34–(–0.05)) and sperms with normal morphology (r = –0.2, 95% CI: –0.36–0.02)). significant inverse association between sperm concentration and TSC with concentrations

					of DMP (β = –0.13, β = –0.12), DMTP (β = –0.04, β = –0.05), and DMDTP (β = –0.81, β = –0.94) and ∑DAP (β = –0.003, β = –0.003) significant inverse association between % of motile sperm and DMTP (β = –0.02), DMDTP (β = –0.44) and DEP (β = –0.06) significant inverse association between TMC with urinary DMP (β = –0.11) and DMDTP (β = –0.81) concentrations
Figueroa et al. (2015) [96], United States	cross-sectional	mean unadjusted metabolite: DMP = 11 ng/ml DMTP = 9 ng/ml DMDTP = 1 ng/ml DEP = 4 ng/ml DETP = 2 ng/ml DEDTP = 0.1 ng/ml CR-adjusted: DMP = 8 ng/ml DMTP = 7 ng/ml DMDTP = 1 ng/ml DEP = 2 ng/ml DETP = 1 ng/ml DEDTP = 0.1 ng/ml	urinary concentration of DMP, DMTP, DMDTP, DEP, DETP, DEDTP, ∑DAP, ∑DEAP, ∑DMAP LODs ranged 0.1–0.6 ng/ml single urine sample collected	159 subfertile men recruited through Massachusetts General Hospital Fertility Center	significant positive association between increasing IRRs by exposure quartiles of DMTP, DMDTP, DEP and DETP with XX18, YY18, XY18 and total disomy highest significant association between the 3rd exposure quartile of DMTP (2.21–6.47 ng/ml) and XX18: IRRQ3 = 1.52, 95% CI: 1.36–1.69 inverse associations between DMP and XX18, XY18 and total sex chromosome disomy inverse associations between DEDTP and YY18, XY18, and total sex chromosome disomy
Pyrethroids					
Perry et al. (2007) [70], China	cross-sectional pilot study	geometric mean: 3PBA = 1.2 μg/l TDCCA = 0.8 μg/l	urinary concentrations of 3-PBA, TDCCA single urine sample collected	18 men with environmental exposure	no statistically significant association between 3-PBA (–0.2 (95% CI: –1.1–0.7)) and TDCCA (–0.6 (95% CI: –1.5–0.3)) with sperm concentration
Meeker et al. (2008) [68], United States	cross-sectional	median unadjusted: 3-PBA = 0.12 μg/l TDCCA = 0.1 μg/l	urinary concentrations of 3-PBA, CDCCA, TDCCA LOD = 0.1 mg/l for all metabolites single urine sample collected	207 men with idiopathic infertility recruited through infertility clinic at Massachusetts	3-PBA concentration > 75th percentile associated with a suggestive 20.2 million sperm/ml reduction (95% CI: –37.1–2.6) in sperm concentration compared with men below the 3-PBA median

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
					significant inverse associations between TDCCA and sperm motility ($p = 0.01$) and sperm motion VSL ($p = 0.02$), VCL ($p = 0.04$), LIN ($p = 0.008$) (after adjusting for CDCCA) dose–response association between 3-PBA and increased sperm DNA damage measured as tail% ($p = 0.02$)
Xia et al. (2008) [73], China	retrospective case-control study	median CR-adjusted 3-PBA = 0.879 μg/g CR	urinary concentrations of 3-PBA levels the concentrations of CR adjusted 3-PBA were categorized into 4 quartiles according to IQR single urine sample collected	376 men with idiopathic infertility recruited through hospitals affiliated to Nanjing Medical University	weak dose–response relationships between 3-PBA levels and decreased sperm concentration ORs (95% CI): II quartile – 1.31 (0.65–2.64), III quartile – 1.73 (0.87–3.45), IV quartile – 2.04 (1.02–4.09) (p for trend 0.027) positive correlations between VCL ($p = 0.039$), VSL ($p = 0.003$) and 3-PBA levels
Ji et al. (2011) [69], China	cross-sectional	median 3-PBA = 1.12 μg/l	urinary concentrations of 3-PBA levels single urine sample collected	240 men with idiopathic infertility recruited through hospitals affiliated to Nanjing Medical University	inverse correlation between 3-PBA urinary level and the sperm concentration ($\beta = -0.27$, 95% CI: –0.41–(–0.12), $p < 0.001$) positive correlation between 3-PBA level and sperm DNA fragmentation ($\beta = 0.27$, 95% CI: 0.15–0.39, $p < 0.001$)
Toshima et al. (2012) [72], Japan	cross-sectional pilot study	mean SG-corrected 3-PBA = 0.547 ng/ml	urinary concentrations of 3-PBA levels LOD = 0.04 ng/ml single urine sample collected	42 men recruited through infertility clinic	inverse significant association between 3-BPA concentration and sperm motility $\beta = -0.374$, $p < 0.01$
Young et al. (2013) [75], United States	cross-sectional	geometric mean unadjusted: 3-PBA = 0.18 μg/l CDCCA = 0.12 μg/l TDCCA = 0.18 μg/l	urinary concentrations of 3-PBA, CDCCA, TDCCA levels 3-BPA LOD = 0.1 μg/l CDCCA LOD = 0.23 μg/l	75 men with idiopathic infertility recruited through infertility clinic	association between CDCCA and TDCCA concentrations above the LOD and increased risk of aneuploidy ranging between 7–30%

		geometric mean SG-adjusted: 3-PBA = 0.24 µg/l CDCCA = 0.15 µg/l TDCCA = 0.23 µg/l geometric mean CR-adjusted: 3-PBA = 0.15 µg/g CR CDCCA = 0.1 µg/g CR TDCCA = 0.14 µg/g CR	TDCCA LOD = 0.35 µg/l single urine sample collected	in Massachusetts	for CDCCA > LOD total disomy (IRR = 1.12 (95% CI: 1.06–1.17)) for TDCCA > LOD total disomy (IRR = 1.09 (95% CI: 1.04–1.15)) association between 3-BPA and aneuploidy non consistent
Imai et al. (2014) [71], Japan	cross-sectional	geometric mean 3-PBA: unadjusted = 0.679 ng/ml SG-adjusted = 0.588	urinary concentrations of 3-PBA levels LOD = 0.08 ng/ml single urine sample collected	322 university healthy students recruited in Metropolitan Tokyo	no significant association between 3-PBA concentration and semen quality parameters (p > 0.05)
Radwan et al. (2015) [76], Poland	cross-sectional	geometric mean unadjusted: CDCCA = 0.12 µg/l TDCCA = 0.16 µg/l 3PBA = 0.17 µg/l DBCA = 0.05 µg/l CR-adjusted: CDCCA = 0.1 µg/g CR TDCCA = 0.15 µg/g CR 3PBA = 0.16 µg/g CR DBCA = 0.04 µg/g CR	urinary concentrations of 3-PBA, CDCCA, TDCCA, DBCA levels LOD = 0.1 ng/ml for all metabolites single urine sample collected	195 men with normal semen concentration[b] and slightly oligozoospermic[c] recruited through infertility clinic in Łódź	association between: CDCCA concentration > 50th percentile with disomy of chromosome 18 (p = 0.05); TDCCA concentration > 50th percentile with XY disomy (p = 0.04) and chromosome 21 disomy (p = 0.05) 3-PBA concentration ≤ 50 and > 50th percentile with XY disomy (p = 0.05 and p = 0.02, respectively), Y disomy (p = 0.04 and p = 0.02), chromosome 21 disomy (p = 0.04 and p = 0.04) and total disomy (p = 0.03 and p = 0.04) 3-PBA concentration > 50th percentile positively associate with chromosome 18 disomy (p = 0.03)
Jurewicz et al. (2015) [67], Poland	cross-sectional	geometric mean unadjusted: CDCCA = 0.12 µg/l TDCCA = 0.16 µg/l 3PBA = 0.17 µg/l DBCA = 0.05 µg/l CR-adjusted: CDCCA = 0.11 µg/g CR TDCCA = 0.15 µg/g CR 3-PBA = 0.16 µg/g CR DBCA =0.04 µg/g CR	urinary concentrations of 3-PBA, CDCCA, TDCCA, DBCA levels single urine sample collected	286 men with normal sperm concentration 4 recruited through infertility clinic in Łódź	positive association between: CDCCA concentration > 50th percentile and the percentage of M DFI and percentage of HDS (p = 0.04 and p = 0.04, respectively) 3-PBA concentration > 50th with percentage of H DFI (p = 0.03)

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
Jurewicz et al. (2016) [74], Poland	cross-sectional	median: 3-PBA = 0.15 µg/l TDCCA = 0.15 µg/l CDCCA = 0.12 µg/l	urinary concentrations of 3-PBA, CDCCA, TDCCA levels LOD = 0.1 ng× ml^{-1} for all metabolites single urine sample collected	194 men with normal sperm concentration[b] and slightly oligozoospermic[c] recruited through infertility clinic in Łódź	negative associations between the concentration CDCCA to TDCCA with Y:X sperm chromosome ratio ($p < 0.001$) in separate models: concentration of CDCCA decrease Y:X ratio ($p = 0.002$) concentration of TDCCA increase the Y:X ratio ($p = 0.003$)
Phthalates					
Pant et al. (2011) [112], India	cross-sectional	mean DEHP: oligoasthenospermic group – 13.47 µg/ml asthenospermic group – 4.11 µg/ml fertile group – 0.80 µg/ml	total semen phthalate concentration of DBP, DEHP LOD = 0.01 mg/ml for DBP, DEHP single semen sample collected	180 healthy young men recruited through Medical University	significant negative association between sperm motility and DEHP in oligoasthenospermic[e] and asthenospermic[f] men ($r = –0.3, –0.25$; $p < 0.001, p < 0.01$, respectively) significant negative association between sperm motility and DBP in oligoasthenospermic and asthenospermic men ($r = –0.25, –0.2$; $p < 0.01, p < 0.01$, respectively)
Toshima et al. (2012) [72], Japan	cross-sectional pilot study	median SG-corrected: MMP = 7.22 ng/ml MEP = 10.7 ng/ml MnBP = 65.7 ng/ml MBzP = 9.18 ng/ml MEHP = 5.94 ng/ml MEHHP = 11.5 ng/ml MEOHP = 7.93 ng/ml 3-PBA = 1.14 ng/ml	urinary concentrations of MMP, MEP, MnBP MBzP, MEHP, MEHHP, MEOHP LODs = 0.006–0.2 ng/ml single urine sample collected	42 men recruited through infertility clinic	significant positive association between MnBP concentration and sperm concentration ($\beta = 0.294$, $p < 0.05$)
Liu et al. (2012) [108], China	cross-sectional	mean unadjusted: MMP = 26.9 ng/ml MEP = 175 ng/ml MBP = 25.7 ng/ml MBzP = 0.42 ng/ml	urinary concentration (free plus conjugated species) of MMP, MEP, MBP, MBzP, MEHP, MEOHP LODs = 0.15–1 µg/l 2 urine samples collected	97 male partners of couples recruited through fertility clinic	significant dose–response relationship between MBP and sperm concentration (ORs for increasing exposure tertiles: 6.8 and 12; p for trend = 0.05)

		MEHP = 1.63 ng/ml MEOHP = 2.7 ng/ml mean CR-adjusted: MMP = 41.3 µg/g MEP = 300 µg/g MBP = 41 µg/g MBzP = 0.78 µg/g MEHP = 2.99 µg/g MEOHP = 3.9 µg/g			significant positive correlation between Cr-adjusted MEP and VSL ($r = 0.232$, $p < 0.05$)
Joensen et al. (2012) [119], Denmark	cross-sectional	median: MEP = 78 ng/ml MnBP = 28 ng/ml MiBP = 58 ng/ml MBzP = 34 ng/ml MEHP = 4 ng/ml MEHHP = 23 ng/ml MEOHP = 14 ng/ml MECPP = 15 ng/ml MOP = 0.1 ng/ml MCPP = 5.0 ng/ml MiNP = 0.6 ng/ml MHiNP = 4.5 ng/ml MOiNP = 2.3 ng/ml MCiOP = 7.7 ng/ml	urinary concentration of 14 phthalate metabolites: MEP, MiBP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MOP, MCPP, MiNP, MHiNP, MOiNP, MCiOP, % MEHP, % MiNP LODs = 0.05–0.63 ng/ml single urine sample collected	881 men from the general population	negative significant association between urinary concentration of MBzP with total sperm count (23% lower in the highest vs. lowest MBzP quartile, 95% CI: –45–(–1)%, $p = 0.04$)
Jurewicz et al. (2013) [113], Poland	cross-sectional	geometric mean: 5OH-MEHP = 24.5 µg/l MEHP = 18.4 µg/l MBzP = 8.3 µg/l MBP = 108.5 µg/l MEP = 153.6 µg/l MiNP = 1.4 µg/l	urinary concentrations of 5OH-MEHP, MEHP, MEP, BBzP, MBzP, MiNP, MBP LODs = 0.01–0.07 µg/l single urine sample collected	269 men with normal sperm concentration[b] and slightly oligozoospermic[c] recruited through infertility clinic in Łódź	significant negative association between MBP with a decrease in CASA parameters: VSL ($p = 0.007$), VCL ($p = 0.009$) and increase sperm DNA damage ($p = 0.047$) significant negative association between percentage of motile sperm cells with 5OH-MEHP ($p = 0.003$), MEHP ($p = 0.001$), MiNP ($p = 0.033$) in multivariate models MBzP positively related to the lack of chromosome 21 ($p = 0.008$), total copy number changes of chromosome 21 ($p = 0.018$)

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
					and chromosome 18 (p = 0.046) significant association between MBP and MEP with increase the lack of chromosome 21 and additional chromosome 18 (p = 0.010 and p = 0.007, respectively) significant association between MEHP and additional chromosome 13 (p = 0.01), the lack of chromosome X or Y (p = 0.011) and total copy number changes of chromosome XY (p = 0.005)
Kranvogl et al. (2014) [109], Slovenia	cross-sectional	mean: DMP = 0.091 µg/l DEP = 1.668 µg/l DBP = 2.546 µg/l BzBP = 2.093 µg/l DEHP = 4.205 µg/l MEP = 0.444 µg/l MnBP = 0.422 µg/l MiBP = 0.403 µg/l MEHP = 0.479 µg/l MiNP = 0.116 µg/l MnOP = 0.075 µg/l MBzP = 0.284 µg/l MEOHP = 0.073 µg/l MEHHP = 0.084 µg/l	urinary concentrations of 5 dialkyl phthalates: DMP, DEP, DBP BzBP, DEHP and 9 phthalate monoesters: MEP, MiBP, MnBP, MEHP, MBzP, MiNP, MnOP, MEOHP, MEHHP LOQs = 0.3–12 µg/l single urine sample collected	136 male partners of couples recruited through fertility clinic	significant negative correlations between sperm concentration and MEHP (β = –0.188, p < 0.05), DMP (β = –0.181, p < 0.05), DBP (β = –0.214, p < 0.05), DEHP (β = –0.266, p < 0.01), MEOHP (β = –0.190, p < 0.05), ∑DEHP (β = –0.302, p < 0.01) significant positive association between semen motility and MEHP (β = –0.224, p < 0.05), DBP (β = –0.204, p < 0.05), MEOHP (β = –0.172, p < 0.05), ∑DEHP (β = –0.234, p < 0.01)
Specht et al. (2014) [118], Greenland, Poland, Ukraine	cross-sectional	mean: proxy-MiNP = 0.004 ng/ml proxy-MEHP = 0.01 ng/ml 5OH-MEHP = 1.2 ng/ml 5oxo-MEHP = 0.2 ng/ml 5cx-MEPP = 1.6 ng/ml 7OH-MMeOP = 0.4 ng/ml 7oxo-MMeOP = 0.04 ng/ml 7cx-MMeHP = 0.8 ng/ml	serum concentration of proxy-MEHP, 5OH-MEHP, oxo-MEHP, 5cx-MEPP, 7OH-MMeOP, 7oxo-MMeOP, 7cx-MMeOP, proxy-MiNP LODs = 0.03–0.2 ng/ml single venous blood sample collected	589 male partners of pregnant women	significant inverse association between proxy-MEHP with semen volume –0.09 (95% CI: –0.15–(–0.02)) and TSC = –0.15 (95% CI: –0.23–(–0.01)) significant inverse association between 5OH-MEHP with semen volume –0.09 (95% CI: –0.15–(–0.02)) and TSC = –0.13

					(95% CI: –0.25–(–0.00)) significant inverse association between 7OH-MMeOP with TSC = –0.06 (95% CI: –0.12–(–0.00))
Pant et al. (2014) [111], India	cross-sectional	mean: DEP = 0.9 μg/ml DBP = 0.97 μg/ml DEHP = 0.59 μg/ml	seminal concentration of DEHP, DBP, DEP LOD for all = 1 ppb single semen sample collected	60 male partners of couples recruited through fertility clinic	association between concentration of DEHP with sperm motility (β = –21.63, p < 0.004), and sperm concentration (β = –17.83, p < 0.001) inverse association between DEHP with normal sperm morphology (p < 0.001) association between DEHP with % DNA in the tail (β = 10.39, p < 0.003), TDM (β = 8.13, p < 0.005), tail length (β = 11.72, p < 0.03)
Han et al. (2014) [117], China	cross-sectional	geometric mean unadjusted: MBP = 17.7 μg/l MEP = 5.3 μg/l MEHP = 4.3 μg/l PA = 1.71 μg/l total PA = 84.48 μg/l CR adjusted: MBP = 22.9 μg/g CR MEP = 6.5 μg/g CR MEHP = 5.4 μg/g CR PA = 2.20 μg/g CR total PA = 114.41 μg/g CR	urinary concentration of MEP, MEHP, MBP, MBzP, PA and total PA LOD = 0.3–1.5 μg/ml single urine sample collected	232 men from general population from the urban area of Chongqing	association between urinary MBP concentration and sperm concentration 1.97 (95% CI: 0.97–4.04) no significant associations between MEP, MEHP, PA or total PA and any of the semen parameters no significant correlation between phthalate metabolites and comet assay parameters
Wang et al. (2015) [107], China	cross-sectional	median CR-corected: MBP = 0.83 ng/ml MEHHP = 0.72 ng/ml MEP = 0.52 ng/ml MBzP = 0.66 ng/ml MEHP = 0.63 ng/ml MEOHP = 0.61 ng/ml % MEHP = 0.52 ng/ml MMP = 0.26 ng/ml	urinary concentrations of MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP, MOP, % MEHP LODs = 0.01–0.04 ng/ml 2 urine samples collected	509 male partners of sub-fertile couples recruited through infertility clinic	positive dose-response relationships between % MEHP and tail DNA% 5.7% (95% CI: 1.2–10.2%) (p for trend < 0.05)
Axelsson et al. (2015) [114], Sweden	cross-sectional	median unadjusted: MEHP = 2.8 ng/ml MECPP = 15 ng/ml	urinary concentration of MEHP, MECPP, MEHHP, MEOHP, MCiOP, MHiNP, MOiNP, MBP,	314 young men from general population	negative association between MECPP, MEOHP, MEHHP, MBP with progressive sperm motility

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
		MEHHP = 21 ng/ml MEOHP = 9.6 ng/ml MCiOP = 16 ng/ml MHiNP = 8.4 ng/ml MOiNP = 5 ng/ml MBP = 47 ng/ml MBzP = 13 ng/ml MEP =41 ng/ml	MBzP, MEP, % MEHP single urine sample collected		(0.001–0.03, with the largest effect size for urinary MECPP) positive association between MEHP with HDS ($p < 0.05$)
Thurston et al. (2015) [115], United States	cross-sectional	geometric mean: MEHP = 3.61 ng/ml MEHHP = 22.87 ng/ml MEOHP = 12.59 ng/ml MECPP = 32.78 ng/ml MBP = 15.28 ng/ml MiBP = 2.80 ng/ml MCPP = 2.09 ng/ml MBzP = 11.13 ng/ml MEP = 200.96 ng/ml	urinary concentration of MEHP, MEHHP, MEOHP, MECPP, MBP, MiBP, MCPP, MBzP, MEP LOD = 0.2–1.2 ng/ml single urine sample collected	420 partners of pregnant women recruited through the studies for future families	no statistically significant associations between any of the semen parameters and any of the metabolites concentrations, with the exception of inverse relationship between MBzP and sperm motility –1.47 (95% CI: –2.61–(–0.33), $p < 0.05$)
Den Hond et al. (2015) [39], Belgium	case-control	geometric mean: controls – MEHP = 2.6 µg/l 5OH-MEHP = 10.9 µg/l 5oxo-MEHP = 7.7 µg/l MEP = 49.9 µg/l MiBP = 55.4 µg/l MnBP = 18.9 µg/l MBzP = 4.5 µg/l case – MEHP = 2.9 µg/l 5OH-MEHP = 10.4 µg/l 5oxo-MEHP = 7.2 µg/l MEP = 40.5 µg/l MiBP = 55.2 µg/l MnBP = 20.7 µg/l MBzP = 4.6 µg/l	urinary concentration of MEHP, MEHHP, MEOHP, MEP, MnBP, MiBP, MBzP single urine sample collected	120 healthy men recruited through fertility clinic case group – 40 men control group – 80 men	no relationships between exposure to phthalates and sperm quality parameters (all p for trend > 0.05)

Bloom et al. (2015) [116], Belgium	prospective cohort study	geometric mean: MEHP = 1.18 ng/ml MCMHP = 18.5 ng/ml MEHHP = 15.2 ng/ml MEOHP = 6.95 ng/ml MECPP = 20.4 ng/ml MMP = 0.54 ng/ml MEP = 86.4 ng/ml MCPP = 5.56 ng/ml MOP = –0.05 ng/ml MNP = 0 ng/ml MiBP = 4.36 ng/ml MBP = 7.28 ng/ml MCHP = 0.00 ng/ml MBzP = 3.57 ng/ml	urinary concentration of MEHP, MCMHP, MEHHP, MEOHP, MECPP, MMP, MEP, MCPP, MOP, MNP, MiBP, MBP, MCHP, MBzP LOQs = 0.05–1 ng/ml single urine sample collected	473 male partners of couples planning contraception were recruited from 16 counties in Michigan and Texas	significantly inverse association between MCMHP, MEHHP, MBzP, MNP with (respectively) TSC: (β = –2.89, 95% CI: –5.62–(–0.17), β = –2.85, β = –4.96, β = –7.2) concentration: (β = –2.2, β = –1.92, β = –3.09, β = –3.62) urinary phthalates monoesters also were significantly associate with: sperm motility: increase (MEHP) and decrease (MCMHP, MEOHP, MECPP, MMP, MCPP); altered morphology (MCMHP, MEHHP, MECPP, MMP, MEP, MNP, MiBP, MBP) altered sperm head (MCMHP, MEHHP, MECPP, MMP, MiBP, MBzP) (all p for trends < 0.05)
Wang et al. (2015) [106], China	cross-sectional	median: 1st urine sample – MMP = 20.78 ng/ml MEP = 18.48 ng/ml MBP = 69.89 ng/ml MBzP = 2.92 ng/ml MEHP = 5.79 ng/ml MEHHP = 13.86 ng/ml MEOHP = 7.92 ng/ml MOP = 0.03 ng/ml % MEHP = 22.1 ng/ml 2nd urine sample – MMP = 21.74 ng/ml MEP = 17.86 ng/ml MBP = 62.46 ng/ml MBzP = 2.95 ng/ml MEHP = 5.68 ng/ml MEHHP = 12.46 ng/ml MEOHP = 7.17 ng/ml MOP = 0.03 ng/ml % MEHP = 23.68 ng/ml	urinary concentration of MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP, MOP LODs = 0.01–0.04 ng/ml 2 urine sample collected	1 040 male partners of sub-fertile couples recruited through infertility clinic	significant inverse associations between MBP and below-references sperm concentration OR comparing extreme MBP quartiles 2.01 (95% CI: 1.07–3.79, p for trend = 0.06) and TSC = 1.8 (95% CI: 1.05–3.08, p for trend 0.02) significant positive associations between MEHP and % of DEHP execrated as MEHP (% MEHP) and the % of abnormal heads (both p for trend < 0.01)

Table 1. Results of studies investigating environmental exposure to endocrine disruptors and associations with semen parameters – cont.

Endocrine disruptor and study	Study design	Concentration of endocrine disrupting chemicals (EDC)	Samples measured	Study population	Results
Pan et al. (2016) [110], China	cross-sectional	geometric mean unadjusted: MMP = 16.9 ng/ml MEP = 14.1 ng/ml MBP = 89.5 ng/ml MiBP = 47.6 ng/ml MCPP = 1.0 ng/ml MEHP = 4.2 ng/ml MEHHP = 12.4 ng/ml MEOHP = 7.7 ng/ml MECPP = 17 ng/ml MCMHP = 4.6 ng/ml MBzP = 0.1 ng/ml MiNP = 0.2 ng/ml MCiOP = 1.6 ng/ml	urinary concentrations of MMP, MEP, MCPP, MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCMHP, MiNP, MCiOP and low-MWP, high-MWP, ∑PAEs LOQ = 0.2 ng/ml for MMP, MBP, MiBP LOQ = 0.1 ng/ml for the other phthalate metabolites single urine sample collected	562 male partners of sub-fertile couples recruited through infertility clinic	significant inverse associations of low-MWP (–19; 95% CI: –26.4–(–11.5), $p < 0.001$), high-MWP (–8.9; 95% CI: –15.4–(–2.4), $p = 0.007$), and ∑PAEs (–18.9; 95% CI: (–26.4–(–11.4); $p < 0.001$) with sperm concentration significant inverse associations of low-MWP (–53.5; 95% CI: –79.5–(–27.6), $p < 0.001$), high-MWP (–22.5; 95% CI: –44.9–(–0.1), $p = 0.049$), and ∑PAEs (−52.3; 95% CI: –78.3–(–26.2), $p < 0.001$) with TSC significant inverse associations of low-MWP (–0.37%; 95% CI: –0.66–(–0.08), $p = 0.012$), high-MWP (–0.31%; 95% CI: –0.56–(–0.06), $p = 0.014$), and ∑PAEs (–0.38%; 95% CI: –0.67–(–0.09), $p = 0.010$) with normal sperm morphology
Wang et al. (2016) [105], China	cross-sectional	mean: MMP = 5.8 µg/l MEP = 2.3 µg/l MBP = 1.2 µg/l MBzP = 0.091 µg/l MEHP = 2.2 µg/l MEHHP = 0.25 µg/l MEOHP = 0.055 µg/l MOP = 0.031 µg/l	semen concentrations of MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP, MOP and % MEHP LODs = 8–43 ng/l single semen sample collected	687 male partners of sub-fertile couples recruited through reproductive center in Wuhan	significant dose-response relationships between decreasing semen volume and MBP 17% (95% CI: –26–(–9.2)%), MEHP 10% (95% CI: –19–(–2.7)%), MEHHP 8.1% (95% CI: –16–(–0.7)%) and MEOHP 18% (95% CI: –26–(–9.4)%) (all p for trend < 0.05) inverse significant associations of MBzP with VCL and VSL, and the associations of MEHP and % MEHP with VCL ($p < 0.05$) suggestive associations of MBzP with percentages of abnormal heads and tails remained ($p = 0.08$, $p = 0.06$, respectively)

Jurewicz et al. (2016) [74], Poland	cross-sectional	geometric mean: MBzP = 6.9 μg/l MBP = 81.9 μg/l MEHP = 15.1 μg/l 5OH MEHP = 19.5 μg/l MEP = 130 μg/l MiNP = 1.2 μg/l	urinary concentrations of 5OH-MEHP, MEHP, MEP, BBzP, MBzP, MINP, MBP LODs ranged 0.01–0.07 μg/l single urine sample collected	194 men with normal sperm concentration[b] and slightly oligozoospermic[c] recruited through fertility clinic in Łódź	negative association between the concentration of 5OH MEHP with Y:X sperm chromosome ratio ($p = 0.033$)

%MEHP –percentage of measured DEHP metabolites excreted as MEHP; ∑DAP – sum of DMP, DMTP, DMDTP, DEP, DETP, DEDTP; ∑DEAP – sum of DEP, DETP and DEDTP metabolites; ∑DEHP – parameter calculated from DEHP, MEHP, MEOHP and MEHHP; ∑DMAP – sum of DMP, DMTP and DMDTP metabolites; ∑PAEs – defined as the sum of MMP, MEP, MCPP, MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCMHP, MiNP, MCiOP; 3-PBA – 3-phenoxybenzoic acid; 5cx-MEPP – 5-carboxy-mono-2-ethylpenty phthalate; 5OH-MEHP – 2-ethyl-5-hydroxy-hexyl phthalate; 5oxo-MEHP – 2-ethyl-5-oxyhexyl phthalate; 7cx-MMeHP – mono(4-methyl-7-carboxyheptyl) phthalate; 7OH-MMeOP – mono-4-methyl-7-hydroxy-octyl phthalate;
7oxo-MMeOP – mono(4-methyl-7-oxooctyl) phthalate; BzBP – benzyl-butyl phthalate; BP – butyl paraben; BzBP – benzyl-butyl phthalate; CDCCA – cis-3-(2,2-dichlorovinyl)-2,2- dimethylcyclopropane carboxylic acid; CR – creatinine; DBCA – cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid; DBP – di-butyl phthalate; DEDTP – diethyldithiophosphate; DEHP – di(2-ethyl- hexyl) phthalate; DEP – di-ethyl phthalate; DETP – diethylthiophosphate; DMDTP – dimethyldithiophosphate; DMP – di-methyl phthalate; DMTP – dimethylthiophosphate; H DFI – high DNA fragmentation index; HDS – high DNA stainability; high-MWP – defined as the sum of MCPP, MEHP, MEHHP, MEOHP, MECPP, MCMHP, MBzP, MiNP and MCiOP, low-MWP – defined as the sum of MMP, MEP, MBP and MiBP, M DFI – medium DNA fragmentation index; MBP – monobutyl phthalate; MBzP – monobenzyl phthalate; MCHP – monocyclohexyl phthalate; MCiOP – mono(carboxy-iso-octyl) phthalate; MCMHP – mono-[(2-carboxymethyl)hexyl] phthalate; MCPP – mono (3-carboxypropyl) phthalate; MECPP – mono (2-ethyl-5-carboxypentyl); MEHHP – mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP – mono(2-ethylhexyl) phthalate; MEOHP – mono (2-ethyl-5-oxohexyl); MEP – monoethyl phthalate; MHiNP – mono-(hydroxy-iso-nonyl) phthalate; MiBP – mono (2-isobutyl) phthalate; MiNP – monoisononylphthalate; MMP – monomethyl phthalate; MnBP – mono-n-butyl phthalate; MnOP – mono-n-octyl phthalate; MNP – mono-isononyl phthalate; MOiNP – mono-(oxo-iso-nonyl) phthalate; MOP – monooctyl phthalate; MP – methylparaben; PA – phthalic acid; PNP – para-nitrophenol; PP – propylparaben; proxy-MEHP – summed 5cx-MEHP, 5OH-MEHP and 5oxo-MEHP according to their molar weight; proxy-MiNP – mono-isononyl; SG – specific gravity; tail% – percent DNA located in the tail; TDCCA – trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; TDCPP – tris(1,3-dichloro-2-propyl) phosphate; TDM – tail distributed moment; TPP – triphenyl phosphate; TSC – total sperm count; VCL – curvilinear velocity; VSL – straight-line velocity.
CASA – computer-aided semen analysis; CI – confidence interval; IQR – interquartile range; IRR – incidence rate ratio; LOD – limit of detection; OR – odds ratio.

[a] Men divided into 4 groups according to spermiogram (World Health Organization 2010 criteria [119]): group 1 – normospermic men, group 2 – oligospermic/asthenospermic/oligoasthenospermic men, group 3 –teratospermic/oligoteratospermic/oligoasthenoteratospermic men, group 4 – azoospermic men.

[b] Semen concentration of 20–300 mln/ml × 10^6 ml^{-1}.

[c] Semen concentration of 15–20 mln/ml × 10^6 ml^{-1}.

[d] Normal semen concentration of 15–300 mln/ml × 10^6 ml^{-1}.

[e] Oligoasthenospermic men according to spermiogram (World Health Organization 1999 criteria [120]).

[f] Asthenospermic men according to spermiogram (World Health Organization 1999 criteria [120]).

One of the most recent studies found associations between semen phthalate metabolites with a decrease in: semen volume (mono-n-butyl phthalate (MBP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)); motion parameters: curvilinear velocity (VCL) (monobenzyl phthalate (MBzP), MEHP, the percentage share of DEHP metabolites excreted as MEHP (% MEHP)) and straight-line velocity (VSL) (MBzP, MEHP, % MEHP) as well as increased percentage share of abnormal heads and tails (MBzP) [103]. An earlier publication by the same authors reported that urinary concentrations of monobutyl phthalate (MBP) was associated with decreases in sperm concentration and total sperm count. Urinary levels of mono-(2-ethylhexyl) phthalate (MEHP) were also linked to an increased percentage share of abnormal heads [104]. The study also investigated whether environmental exposure to phthalates contributed to sperm DNA damage and found a positive dose-response relationship between phthalate metabolites and tail DNA% [105].

In 4 other studies, a significant positive association between urinary phthalate metabolites and sperm concentration was observed [70,106–108]. Another finding in these studies was a significant inverse relationship between urinary phthalate metabolites on the one hand and total sperm count, sperm morphology [108], sperm motility [107] and sperm motion parameters VSL on the other [106]. Pant et al. (2014) examined seminal concentrations of phthalates and found that di(2-ethylhexyl) phthalate (DEHP) might contribute to a decline in semen quality parameters such as sperm motility, concentration and morphology, and that it could induce DNA damage [109].

Three years earlier the same authors reported a significant negative association between sperm motility and the seminal concentrations of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) [110]. These results are consistent with the findings of the study performed among Polish subfertile males, in the case of which the levels of urinary phthalate metabolites were significantly associated with a decrease in sperm motility (mono (2-ethyl-5-hydroxyhexyl)phthalate (5OH MEHP), MEHP, monoisononylphthalate (MiNP)), computer-aided semen analysis (CASA) parameters (mono-n-butyl phthalate (MBP)), and an increase in sperm DNA damage (MBP). The authors found that phthalate exposure was also associated with sperm aneuploidy (MBzP, MBP, MEHP, monoethylphthalate (MEP)) [111].

In another study by the same authors, a negative association between the concentration of 5OH MEHP and Y:X sperm chromosome ratio was observed [72]. This review identified only one study performed among subfertile patients in which no relationship between the exposure to phthalates and sperm quality parameters was observed [39]. Furthermore, all the studies performed among men from the general population have reported an association between the exposure to phthalates and semen quality. One of the recent studies conducted among young healthy men from Sweden reported that levels of DEHP metabolites were associated with a lower proportion of progressively motile and mature spermatozoa [112].

Two other studies found a link between other urinary phthalates metabolites and sperm motility [113,114]. Bloom et al. (2015) also showed a significant inverse association of phthalates with sperm concentration, total sperm count, sperm morphology and sperm head [114]. Additionally, Han et al. (2014) reported an association between urinary MBP concentration and sperm concentration [115]. Another study, in which serum concentrations of phthalate metabolites were measured, reported that an increasing exposure to DEHP primary metabolite (Proxy-MEHP) and 5OH-MEHP was associated with decreased semen volume and total sperm count [116]. Joensen et al. (2012) also found a negative significant association between urinary concentrations of phthalate metabolites and one of the semen quality parameters. The study

revealed a negative significant association between urinary concentration of MBzP and total sperm count [117].

The results of the reviewed studies indicate the same association between environmental exposure to phthalates and semen quality outcomes.

CONCLUSIONS

Most of the studies reviewed in this paper showed an association between exposure biomarkers and non-persistent EDCs and at least one parameter of semen quality (Table 1).

Sperm concentration was the most frequently measured parameter. In most of the reviewed studies, a positive correlation between the investigated EDC and this parameter was found. This association was therefore found in all the studies in which urinary concentrations of OP metabolites and sperm concentrations were measured [73,93–95]. The same correlation was also found in most studies of BPA [19,20,23–25] and phthalates [70,104,106–109,114,115]. There is also some evidence that non-persistent EDCs may impact sperm motion parameters [68,71,103,106,111]. Another parameter which was measured in almost all the studies was semen volume and total sperm count. The results were, however, inconsistent (Table 2). Inconsistent results, with a predominance of negative outcomes, were also found in numerous studies in which progressive motility, total motile count and morphology after EDC exposure were assessed (Table 2).

Studies have been consistent about the impact of synthetic pyrethroids, OPPs and phthalates on sperm aneuploidy and X:Y ratio. However, there were only 5 studies which assessed these parameters [72,75,76,96,111]. In almost all of the reviewed papers, there was an association between the exposure to some non-persistent EDCs (BPA, parabens, pyrethroids, phthalates) and DNA damage measured as tail% and DFI (Table 2). In only 2 of the 11 studies in which this parameter was measured, no such associations were found [112,115].

The inconsistencies in the results may be due to many limitations. In the case of studies investigating BPA exposure, the variety of results may have been due to the differences in the selection of study groups and biological fluids in which concentrations of BPA were measured. In TCS studies, the use of diverse methods of urinary TCS quantification and various statistical models may have affected the results. The divergence of the results in the studies of phthalates exposure and semen quality may have arisen from the various confounding factors identified in the studies, the creatinine adjustment or specific gravity adjustment, the different sample size and the different phthalate metabolites assessed. To the best of our knowledge, the relationship between TCS exposure and semen quality was only assessed in one study, so it is difficult to compare the results. The studies showed that the exposure to synthetic pyrethroids and OPPs was associated with a negative impact on semen quality.

In general, the limitations of the studies could have arisen from the study design, as most of the studies were cross-sectional studies. This type of study is known to have several shortcomings, including the limitation in causal inference. Only 6 prospective cohort studies, which do not have this bias, were identified [18,20,22,25,73,114]. The study sample size and the varying definitions of exposure could also have impacted the results. The use of various biomarkers to ascertain exposure may have some bearing on the statistical association. In most of the studies, concentrations of endocrine disruptor metabolites in urine were measured. In several publications, the selected chemicals were also measured in semen and plasma [23,24,103,109,110,116]. Only one study assessed the parent compounds [94]. Additionally, the various endpoints for the assessment of semen quality may be a possible explanation for the differences in the study's results.

Another limitation is the difficulty to determine the timing of exposure to EDCs in relation to spermatogenesis (which lasts about 70–80 days). In most of the studies,

Table 2. Summary of associations of environmental exposure to endocrine disruptors with semen quality

Semen parameters	Association					
	bisphenol A	triclosan	parabens	synthetic pyrethroids	organophosphorus pesticides	phthalates
Sperm concentration	(–) Mendiola et al. (2010) [18] (+) Meeker et al. (2010) [19] (+) Li et al. (2011) [25] (+) Knez et al. (2014) [20] (–) Lassen et al. (2014) [21] (–) Goldstone et al. (2015) [22] (+) Vitku et al. (2015) [23] (+) Vitku et al. (2016) [24]	(–) Chen et al. (2013) [38] (+) Zhu et al. (2016) [37]	(–) Meeker et al. (2010) [50]	(–) Perry et al. (2007) [70] (+) Meeker et al. (2008) [68] (+) Xia et al. (2008) [73] (+) Ji et al. (2011) [69] (–) Toshima et al. (2012) [72] (–) Imai et al. (2014) [71]	(+) Perry et al. (2007) [70] (+) Meeker et al. (2010) [94] (+) Perry et al. (2011) [93] (+) Melgarejo et al. (2015) [95]	(–) Pant et al. (2011) [110] (+) Toshima et al. (2012) [72] (+) Liu et al. (2012) [106] (–) Joensen et al. (2012) [117] (–) Jurewicz et al. (2013) [111] (+) Kranvogl et al. (2014) [107] (–) Specht et al. (2014) [116] (+) Pant et al. (2014) [109] (+) Han et al. (2014) [115] (–) Axelsson et al. (2015) [112] (+) Wang et al. (2015) [104] (+) Bloom et al. (2015) [114] (–) Den Hond et al. (2015) [39] (–) Thurston et al. (2015) [113] (+) Pan et al. (2016) [108] (–) Wang et al. (2016) [103]
Sperm volume	(–) Mendiola et al. (2010) [18] (–) Li et al. (2011) [25] (–) Knez et al. (2014) [20] (–) Lassen et al. (2014) [21] (–) Goldstone et al. (2015) [22]	(–) Chen et al. (2013) [38] (–) Zhu et al. (2016) [37]		(–) Xia et al. (2008) [73] (–) Ji et al. (2011) [69] (–) Toshima et al. (2012) [72] (–) Imai et al. (2014) [71]	(–) Melgarejo et al. (2015) [95]	(–) Pant et al. (2011) [110] (–) Joensen et al. (2012) [117] (–) Liu et al. (2012) [106] (–) Toshima et al. (2012) [72] (–) Han et al. (2014) [115] (+) Specht et al. (2014) [116] (–) Wang et al. (2015) [104] (–) Bloom et al. (2015) [114] (–) Axelsson et al. (2015) [112] (+) Wang et al. (2016) [103]
Progressive motility	(–) Mendiola et al. (2010) [18] (+) Meeker et al. (2010) [19] (–) Li et al. (2011) [25] (–) Knez et al. (2014) [20] (+) Lassen et al. (2014) [21] (–) Goldstone et al. (2015) [22] (–) Vitku et al. (2015) [23] (–) Vitku et al. (2016) [24]	(+) Zhu et al. (2016) [37]	(–) Meeker et al. (2010) [50]	(+) Meeker et al. (2008) [68] (–) Xia et al. (2008) [73] (–) Ji et al. (2011) [69] (+) Toshima et al. (2012) [72]	(–) Meeker et al. (2010) [94] (+) Perry et al. (2011) [93] (+) Melgarejo et al. (2015) [95]	(+) Pant et al. (2011) [110] (–) Joensen et al. (2012) [117] (–) Liu et al. (2012) [106] (–) Toshima et al. (2012) [72] (+) Jurewicz et al. (2013) [111] (–) Han et al. (2014) [115] (+) Kranvogl et al. (2014) [107] (–) Wang et al. (2015) [104]

				(–) Imai et al. (2014) [71]		(+) Axelsson et al. (2015) [112] (+) Bloom et al. (2015) [114] (–) Den Hond et al. (2015) [39] (+) Thurston et al. (2015) [113] (–) Wang et al. (2016) [103] (–) Pan et al. (2016) [108]
Non progressive motility	(–) Knez et al. (2014) [20]					
Total motile count	(–) Mendiola et al. (2010) [18] (+) Knez et al. (2014) [20]	(–) Den Hond et al. (2015) [39]		(–) Imai et al. (2014) [71]	(+) Melgarejo et al. (2015) [95]	(+) Pant et al. (2014) [109] (–) Wang et al. (2015) [104] (–) Thurston et al. (2015) [113] (–) Wang et al. (2016) [103]
Morphology	(–) Mendiola et al. (2010) [18] (+) Meeker et al. (2010) [19] (–) Li et al. (2011) [25] (–) Knez et al. (2014) [20] (–) Lassen et al. (2014) [21] (–) Goldstone et al. (2015) [22] (–) Vitku et al. (2015) [23] (+) Vitku et al. (2016) [24]	(+) Zhu et al. (2016) [37]	(–) Meeker et al. (2010) [50]	(–) Meeker et al. (2008) [68]	(–) Meeker et al. (2010) [94] (+) Melgarejo et al. (2015) [95]	(–) Joensen et al. (2012) [117] (–) Liu et al. (2012) [106] (–) Jurewicz et al. (2013) [111] (–) Han et al. (2014) [115] (+) Pant et al. (2014) [109] (+) Wang et al. (2015) [104] (+) Bloom et al. (2015) [114] (–) Den Hond et al. (2015) [39] (–) Thurston et al. (2015) [113] (–) Axelsson et al. (2015) [112] (+) Wang et al. (2016) [103] (+) Pan et al. (2016) [108]
Total sperm count	(–) Mendiola et al. (2010) [18] (–) Meeker et al. (2010) [19] (+) Li et al. (2011) [25] (+) Knez et al. (2014) [20] (–) Lassen et al. (2014) [21] (–) Goldstone et al. (2015) [22] (+) Vitku et al. (2015) [23] (+) Vitku et al. (2016) [24]	(–) Chen et al. (2013) [38] (+) Zhu et al. (2016) [37]	(–) Meeker et al. (2010) [50]	(–) Xia et al. (2008) [73] (–) Ji et al. (2011) [69] (–) Imai et al. (2014) [71]	(+) Melgarejo et al. (2015) [95]	(+) Joensen et al. (2012) [117] (–) Han et al. (2014) [115] (+) Specht et al. (2014) [116] (+) Wang et al. (2015) [104] (+) Bloom et al. (2015) [114] (–) Thurston et al. (2015) [113] (–) Axelsson et al. (2015) [112] (–) Wang et al. (2016) [103] (+) Pan et al. (2016) [108]
Sperm motion (VSL, VCL, LIN, VAP, BCF, STR)	(–) Meeker et al. (2010) [19]	(–) Zhu et al. (2016) [37]	(–) Meeker et al. (2010) [50]	(+) Meeker et al. (2008) [68] (+) Xia et al. (2008) [73]		(+) Liu et al. (2012) [106] (+) Jurewicz et al. (2013) [111] (–) Wang et al. (2015) [104] (+) Wang et al. (2016) [103]

Table 2. Summary of associations of environmental exposure to endocrine disruptors with semen quality – cont.

Semen parameters	Association					
	bisphenol A	triclosan	parabens	synthetic pyrethroids	organophosphorus pesticides	phthalates
Sperm DNA damage						
comet extent, tail length	(–) Meeker et al. (2010) [19]		(–) Meeker et al. (2010) [50]	(–) Meeker et al. (2008) [68]		(–) Han et al. (2014) [115] (+) Pant et al. (2014) [109] (–) Wang et al. (2015) [105]
TDM	(–) Meeker et al. (2010) [19]		(–) Meeker et al. (2010) [50]	(–) Meeker et al. (2008) [68]		(–) Han et al. (2014) [115] (+) Pant et al. (2014) [109] (–) Wang et al. (2015) [105]
tail%	(+) Meeker et al. (2010) [19]		(+) Meeker et al. (2010) [50]	(+) Meeker et al. (2008) [68]		(–) Han et al. (2014) [115] (+) Pant et al. (2014) [109] (+) Wang et al. (2015) [105]
DFI	(+) Goldstone et al. (2015) [22]			(+) Ji et al. (2011) [69] (+) Jurewicz et al. (2015) [67]		(+) Jurewicz et al. (2013) [111] (–) Axelsson et al. (2015) [112]
HDS				(+) Jurewicz et al. (2015) [67]		(+) Axelsson et al. (2015) [112]
Aneuploidy				(+) Young et al. (2013) [75] (+) Radwan et al. (2015) [76]	(+) Figueroa et al. (2015) [96]	(+) Jurewicz et al. (2013) [111]
X:Y ratio				(+) Jurewicz et al. (2016) [74]		(+) Jurewicz et al. (2016) [74]

(–) – no significant association; (+) – significant association.
VSL – straight-line velocity; VCL – curvilinear velocity; LIN – linearity; VAP – average path velocity; BCF – beat cross frequency; STR – straightness; TDM – tail distributed moment; tail% – percent DNA located in the tail; DFI – DNA fragmentation index; HDS – high DNA stainability.

only one urine sample was collected from each patient and analyzed. As non-persistent endocrine disruptors are metabolized in 24–48 h, a single urine sample may not reliably define the usual exposure and a clear association between exposure and semen parameters is difficult to assess. However, Meeker et al. (2005) have reported that a single sample adequately predicts longer-term average exposure [118]. In addition, in most of the studies, a single semen sample was collected to measure semen parameters. In this case it was also reported that a single sample was enough to correctly evaluate semen parameters [95].

And lastly, the inconsistencies in the results may have been caused by the disparity in exposure levels, uncontrolled confounding factors and differences in the statistical analysis. In some of the studies, a large number of statistical comparisons was made, which cannot eliminate chance findings [50,70]. It should also be noted that co-exposure from other environmental chemicals might have altered the observed associations.

In conclusion, despite the numerous limitations of the results the reviewed studies suggest that exposure to non-persistent endocrine disruptors (bisphenol A, triclosan, parabens, OPPs, pyrethroids and phthalates) may affect semen quality parameters. Due to the insufficient evidence further epidemiological studies are needed to confirm these findings.

REFERENCES

1. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev. 2015;36(6):1–150, https://doi.org/10.1210/er.2015-1010.
2. Kabir ER, Rahman MS, Rahman I. A review on endocrine disruptors and their possible impacts on human health. Environ Toxicol Pharmacol. 2015;40(1):241–58, https://doi.org/10.1016/j.etap.2015.06.009.
3. Sweeney MF, Hasan N, Soto AM, Sonnenschein C. Environmental endocrine disruptors: Effects on the human male reproductive system. Rev Endocr Metab Disord. 2015;16(4):341–57, https://doi.org/10.1007/s11154-016-9337-4.
4. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: An Endocrine Society scientific statement. Endocr Rev. 2009;30(4):293–342, https://doi.org/10.1210/er.2009-0002.
5. Lallas P. The Stockholm convention on persistent organic pollutants. Am J Int Law. 2008;95(3):692–708, https://doi.org/10.2307/2668517.
6. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: A committee opinion. Fertil Steril. 2015;103(3):e18–25, https://doi.org/10.1016/j.fertnstert.2014.12.103.
7. Marques-Pinto A, Carvalho D. Human infertility: Are endocrine disruptors to blame? Endocr Connect. 2013;2:15–29, https://doi.org/10.1530/EC-13-0036.
8. Sengupta P, Dutta S, Krajewska-Kulak E. The disappearing sperms: Analysis of reports published between 1980 and 2015. Am J Mens Health. 2016;11(4):1279–304, https://doi.org/10.1177/1557988316643383.
9. Dodds EC, Lawson W. Synthetic estrogenic agents without the phenanthrene nucleus. Nature. 1936;137(3476):996, https://doi.org/10.1038/137996a0.
10. Teng C, Goodwin B, Shockley K, Xia M, Huang R, Norris J, et al. Bisphenol A affects androgen receptor function via multiple mechanisms. Chem Biol Interact. 2013;203(3):556–64, https://doi.org/10.1016/j.cbi.2013.03.013.
11. Manfo FPT, Jubendradass R, Nantia EA, Moundipa PF, Mathur P. Adverse effects of bisphenol A on male reproductive function. Rev Environ Contam Toxicol. 2014;228:57–82, https://doi.org/10.1007/978-3-319-01619-1_3.
12. National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR). NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol A. NTP CERHR MON. 2008;22(8):1–321
13. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod

Toxicol. 2007;24(2):139–77, https://doi.org/10.1016/j.reprotox.2007.07.010.

14. Rahman MS, Kwon W-S, Lee J-S, Yoon S-J, Ryu B-Y, Pang M-G. Bisphenol-A affects male fertility via fertility-related proteins in spermatozoa. Sci Rep. 2015;5(1):9169, https://doi.org/10.1038/srep09169.
15. Wang H-F, Liu M, Li N, Luo T, Zheng L-P, Zeng X-H. Bisphenol A impairs mature sperm functions by a CatSper-relevant mechanism. Toxicol Sci. 2016;152(1):145–54, https://doi.org/10.1093/toxsci/kfw070.
16. Vilela J, Hartmann A, Silva EF, Cardoso T, Corcini CD, Varela-Junior AS, et al. Sperm impairments in adult vesper mice (*Calomys laucha*) caused by *in utero* exposure to bisphenol A. Andrologia. 2014;46(9):971–8, https://doi.org/10.1111/and.12182.
17. Zhang GL, Zhang XF, Feng YM, Li L, Huynh E, Sun XF, et al. Exposure to bisphenol A results in a decline in mouse spermatogenesis. Reprod Fertil Dev. 2013;25(6):847–59, https://doi.org/10.1071/RD12159.
18. Mendiola J, Jørgensen N, Andersson A-M, Calafat AM, Ye X, Redmon JB, et al. Are environmental levels of bisphenol A associated with reproductive function in fertile men? Environ Health Perspect. 2010;118(9):1286–91, https://doi.org/10.1289/ehp.1002037.
19. Meeker JD, Ehrlich S, Toth TL, Wright DL, Calafat AM, Trisini AT, et al. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reprod Toxicol. 2010;30(4):532–9, https://doi.org/10.1016/j.reprotox.2010.07.005.
20. Knez J, Kranvogl R, Breznik BP, Vončina E, Vlaisavljević V. Are urinary bisphenol A levels in men related to semen quality and embryo development after medically assisted reproduction? Fertil Steril. 2014;101(1):215–21, https://doi.org/10.1016/j.fertnstert.2013.09.030.
21. Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, et al. Urinary bisphenol A levels in young men: Association with reproductive hormones and semen quality. Environ Health Perspect. 2014;122(5):478–84, https://doi.org/10.1289/ehp.1307309.
22. Goldstone AE, Chen Z, Perry MJ, Kannan K, Louis GMB. Urinary bisphenol A and semen quality, the LIFE study. Reprod Toxicol. 2015;51:7–13, https://doi.org/10.1016/j.reprotox.2014.11.003.
23. Vitku J, Sosvorova L, Chlupacova T, Hampl R, Hill M, Sobotka V, et al. Differences in bisphenol A and estrogen levels in the plasma and seminal plasma of men with different degrees of infertility. Physiol Res. 2015;64 Suppl 2:303–11.
24. Vitku J, Heracek J, Sosvorova L, Hampl R, Chlupacova T, Hill M, et al. Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. Environ Int. 2016;89–90:166–73, https://doi.org/10.1016/j.envint.2016.01.021.
25. Li D-K, Zhou Z, Miao M, He Y, Wang J, Ferber J, et al. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil Steril. 2011;95(2):625–30, https://doi.org/10.1016/j.fertnstert.2010.09.026.
26. Johnson PI, Koustas E, Vesterinen HM, Sutton P, Atchley DS, Kim AN, et al. Application of the Navigation Guide systematic review methodology to the evidence for developmental and reproductive toxicity of triclosan. Environ Int. 2016;92:716–28, https://doi.org/10.1016/j.envint.2016.03.009.
27. Halden RU. On the need and speed of regulating triclosan and triclocarban in the United States. Environ Sci Technol. 2014;48(7):3603–11, https://doi.org/10.1021/es500495p.
28. Von der Ohe PC, Schmitt-Jansen M, Slobodnik J, Brack W. Triclosan – The forgotten priority substance? Environ Sci Pollut Res Int. 2012 Feb;19(2):585–91, https://doi.org/10.1007/s11356-011-0580-7
29. Dhillon GS, Kaur S, Pulicharla R, Brar SK, Cledón M, Verma M, et al. Triclosan: Current status, occurrence, environmental risks and bioaccumulation potential. Int J Environ Res Public Health. 2015;12(5):5657–84, https://doi.org/10.3390/ijerph120505657.
30. Wang C-F, Tian Y. Reproductive endocrine-disrupting effects of triclosan: Population exposure, present evidence and

potential mechanisms. Environ Pollut. 2015;206:195–201, https://doi.org/10.1016/j.envpol.2015.07.001.

31. Witorsch RJ. Critical analysis of endocrine disruptive activity of triclosan and its relevance to human exposure through the use of personal care products. Crit Rev Toxicol. 2014;44(6):535–55, https://doi.org/10.3109/10408444.2014.910754.
32. Kumar V, Balomajumder C, Roy P. Disruption of LH-induced testosterone biosynthesis in testicular Leydig cells by triclosan: Probable mechanism of action. Toxicology. 2008;250(2–3):124–31, https://doi.org/10.1016/j.tox.2008.06.012.
33. Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR. BLTK1 murine Leydig cells: A novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. Toxicol Sci. 2012;127(2):391–402, https://doi.org/10.1093/toxsci/kfs121.
34. Kumar V, Chakraborty A, Kural MR, Roy P. Alteration of testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan. Reprod Toxicol. 2009;27(2):177–85, https://doi.org/10.1016/j.reprotox.2008.12.002.
35. Zorrilla LM, Gibson EK, Jeffay SC, Crofton KM, Setzer WR, Cooper RL, et al. The effects of triclosan on puberty and thyroid hormones in male Wistar rats. Toxicol Sci. 2009;107(1):56–64, https://doi.org/10.1093/toxsci/kfn225.
36. Axelstad M, Boberg J, Vinggaard AM, Christiansen S, Hass U. Triclosan exposure reduces thyroxine levels in pregnant and lactating rat dams and in directly exposed offspring. Food Chem Toxicol. 2013;59:534–40, https://doi.org/10.1016/j.fct.2013.06.050.
37. Zhu W, Zhang H, Tong C, Xie C, Fan G, Zhao S, et al. Environmental exposure to triclosan and semen quality. Int J Environ Res Public Health. 2016;13(2):224, https://doi.org/10.3390/ijerph13020224.
38. Chen M, Tang R, Fu G, Xu B, Zhu P, Qiao S, et al. Association of exposure to phenols and idiopathic male infertility. J Hazard Mater. 2013;250–251:115–21, https://doi.org/10.1016/j.jhazmat.2013.01.061.
39. Hond E Den, Tournaye H, Sutter P De, Ombelet W, Baeyens W, Covaci A, et al. Human exposure to endocrine disrupting chemicals and fertility: A case-control study in male subfertility patients. Environ Int. 2015;84:154–60, https://doi.org/10.1016/j.envint.2015.07.017.
40. Błędzka D, Gromadzińska J, Wąsowicz W. Parabens. From environmental studies to human health. Environ Int. 2014;67:27–42, https://doi.org/10.1016/j.envint.2014.02.007.
41. Boberg J, Taxvig C, Christiansen S. Possible endocrine disrupting effects of parabens and their metabolites. Reprod Toxicol. 2010;30(2):301–12, https://doi.org/10.1016/j.reprotox.2010.03.011.
42. Cashman AL, Warshaw EM. Parabens: A review of epidemiology, structure, allergenicity, and hormonal properties. Dermatitis. 2005;16(2):57–66.
43. Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. Concentrations of parabens in human breast tumours. J Appl Toxicol. 2004;24(1):5–13, https://doi.org/10.1002/jat.958.
44. Darbre PD, Harvey PW. Parabens can enable hallmarks and characteristics of cancer in human breast epithelial cells: A review of the literature with reference to new exposure data and regulatory status. J Appl Toxicol. 2014;34(9):925–38, https://doi.org/10.1002/jat.3027.
45. Boberg J, Axelstad M, Svingen T, Mandrup K, Christiansen S, Vinggaard AM, et al. Multiple endocrine disrupting effects in rats perinatally exposed to butylparaben. Toxicol Sci. 2016;152(1):244–56, https://doi.org/10.1093/toxsci/kfw079.
46. Kang K-S, Che J-H, Ryu D-Y, Kim T-W, Li G-X, Lee Y-S. Decreased sperm number and motile activity on the F1 offspring maternally exposed to butyl p-hydroxybenzoic acid (butyl paraben). J Vet Med Sci. 2002;64(3):227–35, https://doi.org/10.1292/jvms.64.227.
47. Zhang L, Dong L, Ding S, Qiao P, Wang C, Zhang M, et al. Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed E_2 levels in male rat offspring. Environ Toxicol Pharmacol. 2014;37(2):705–17, https://doi.org/10.1016/j.etap.2014.01.016.

48. Chen J, Ahn KC, Gee NA, Gee SJ, Hammock BD, Lasley BL. Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. Toxicol Appl Pharmacol. 2007;221(3):278–84, https://doi.org/10.1016/j.taap.2007.03.015.
49. Tavares RS, Martins FC, Oliveira PJ, Ramalho-Santos J, Peixoto FP. Parabens in male infertility – Is there a mitochondrial connection? Reprod Toxicol. 2009;27(1):1–7, https://doi.org/10.1016/j.reprotox.2008.10.002.
50. Meeker JD, Yang T, Ye X, Calafat AM, Hauser R. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ Health Perspect. 2010;119(2):252–7, https://doi.org/10.1289/ehp.1002238.
51. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for pyrethrins and pyrethroids. Atlanta (GA): U.S. Department of Health and Human Services, Public Health Service; 2003.
52. Roberts JR, Reigart JR. Recognition and management of pesticide poisonings. 6th ed. Washington DC: CreateSpace Independent Pub; 2014.
53. Fortes C, Mastroeni S, Pilla MA, Antonelli G, Lunghini L, Aprea C. The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite. Food Chem Toxicol. 2013;52:91–6, https://doi.org/10.1016/j.fct.2012.10.035.
54. Centers of Disease Control and Prevention [Internet]. Atlanta: Centers; 2016 [cited 2016 Sep 1]. Pyrethroid pesticides overview. Available from: https://www.cdc.gov/biomonitoring/Cyfluthrin_Cypermethrin_Permethrin_Biomonitoring-Summary.html.
55. Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett. 2012;210(2):155–68, https://doi.org/10.1016/j.toxlet.2011.10.007.
56. Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O, Roig B. Effect of endocrine disruptor pesticides: A review. Int J Environ Res Public Health. 2011;8(12):2265–303, https://doi.org/10.3390/ijerph8062265.
57. Chen H, Xiao J, Hu G, Zhou J, Xiao H, Wang X. Estrogenicity of organophosphorus and pyrethroid pesticides. J Toxicol Environ Health A. 2002;65(19):1419–35, https://doi.org/10.1080/00984100290071243.
58. Zhang J, Zhu W, Zheng Y, Yang J, Zhu X. The antiandrogenic activity of pyrethroid pesticides cyfluthrin and β-cyfluthrin. Reprod Toxicol. 2008;25(4):491–6, https://doi.org/10.1016/j.reprotox.2008.05.054.
59. Marinowic DR, Mergener M, Pollo TA, Maluf SW, da Silva LB. *In vivo* genotoxicity of the pyrethroid pesticide beta-cyfluthrin using the comet assay in the fish *Bryconamericus iheringii*. Z Naturforsch C. 2012;67(5–6):308–11.
60. Ismail MF, Mohamed HM. Deltamethrin-induced genotoxicity and testicular injury in rats: Comparison with biopesticide. Food Chem Toxicol. 2012;50(10):3421–5, https://doi.org/10.1016/j.fct.2012.07.060.
61. Taju G, Abdul Majeed S, Nambi KSN, Farook MA, Vimal S, Sahul Hameed AS. *In vitro* cytotoxic, genotoxic and oxidative stress of cypermethrin on five fish cell lines. Pestic Biochem Physiol. 2014;113:15–24, https://doi.org/10.1016/j.pestbp.2014.06.006.
62. Zhang H, Wang H, Ji YL, Zhang Y, Yu T, Ning H, et al. Maternal fenvalerate exposure during pregnancy persistently impairs testicular development and spermatogenesis in male offspring. Food Chem Toxicol. 2010;48(5):1160–9, https://doi.org/10.1016/j.fct.2010.02.003.
63. Wang H, Wang Q, Zhao X-F, Liu P, Meng X-H, Yu T, et al. Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes. Arch Toxicol. 2010;84(1):53–61, https://doi.org/10.1007/s00204-009-0479-y.
64. Yuan C, Wang C, Gao SQ, Kong TT, Chen L, Li XF, et al. Effects of permethrin, cypermethrin and 3-phenoxybenzoic acid on rat sperm motility *in vitro* evaluated with computer-assisted sperm analysis. Toxicol in Vitro. 2010;24(2):382–6, https://doi.org/10.1016/j.tiv.2009.11.001.

65. Ahmad M, Hussain I, Khan A, Najib-ur-Rehman. Deleterious effects of cypermethrin on semen characteristics and testes of dwarf goats (*Capra hircus*). Exp Toxicol Pathol. 2009;61(4):339–46, https://doi.org/10.1016/j.etp.2008.10.002.
66. Meeker JD, Barr DB, Hauser R. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol. 2009;27(2):155–60, https://doi.org/10.1016/j.reprotox.2008.12.012.
67. Jurewicz J, Radwan M, Wielgomas B, Sobala W, Piskunowicz M, Radwan P, et al. The effect of environmental exposure to pyrethroids and DNA damage in human sperm. Syst Biol Reprod Med. 2015;61(1):37–43, https://doi.org/10.3109/19396368.2014.981886.
68. Meeker JD, Barr DB, Hauser R. Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Hum Reprod. 2008;23(8):1932–40, https://doi.org/10.1093/humrep/den242.
69. Ji G, Xia Y, Gu A, Shi X, Long Y, Song L, et al. Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in Chinese men. Reprod Toxicol. 2011;31(2):171–6, https://doi.org/10.1016/j.reprotox.2010.10.005.
70. Perry MJ, Venners SA, Barr DB, Xu X. Environmental pyrethroid and organophosphorus insecticide exposures and sperm concentration. Reprod Toxicol. 2007;23(1):113–8, https://doi.org/10.1016/j.reprotox.2006.08.005.
71. Imai K, Yoshinaga J, Yoshikane M, Shiraishi H, Mieno MN, Yoshiike M, et al. Pyrethroid insecticide exposure and semen quality of young Japanese men. Reprod Toxicol. 2014;43:38–44, https://doi.org/10.1016/j.reprotox.2013.10.010.
72. Toshima H, Suzuki Y, Imai K, Yoshinaga J, Shiraishi H, Mizumoto Y, et al. Endocrine disrupting chemicals in urine of Japanese male partners of subfertile couples: A pilot study on exposure and semen quality. Int J Hyg Environ Health. 2012;215(5):502–6, https://doi.org/10.1016/j.ijheh.2011.09.005.
73. Xia Y, Han Y, Wu B, Wang S, Gu A, Lu N, et al. The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. Fertil Steril. 2008;89(6):1743–50, https://doi.org/10.1016/j.fertnstert.2007.05.049.
74. Jurewicz J, Sobala W, Hanke W. Exposure to widespread environmental endocrine disrupting chemicals and human sperm sex ratio. Environ Pollut. 2016;213:732–40, https://doi.org/10.1016/j.envpol.2016.02.008.
75. Young HA, Meeker JD, Martenies SE, Figueroa ZI, Barr DB, Perry MJ. Environmental exposure to pyrethroids and sperm sex chromosome disomy: A cross-sectional study. Environ Health. 2013;12:111, https://doi.org/10.1186/1476-069X-12-111.
76. Radwan M, Jurewicz J, Wielgomas B, Piskunowicz M, Sobala W, Radwan P, et al. The association between environmental exposure to pyrethroids and sperm aneuploidy. Chemosphere. 2015;128:42–8, https://doi.org/10.1016/j.chemosphere.2014.12.077.
77. Bates N, Campbell A. Organophosphate insecticides. In: Campbell A, Chapman M. Handbook of poisoning in dogs and cats. Oxford: Blackwell Science Ltd; 2008. p. 199–204, https://doi.org/10.1002/9780470699010.ch50.
78. Meyer J, Bester K. Organophosphate flame retardants and plasticisers in wastewater treatment plants. J Environ Monit. 2004;6(7):599–605, https://doi.org/10.1039/b403206c.
79. Chowdhary S, Bhattacharyya R, Banerjee D. Acute organophosphorus poisoning. Clin Chim Acta. 2014;431:66–76, https://doi.org/10.1016/j.cca.2014.01.024.
80. Centers of Disease Control and Prevention [Internet]. Atlanta: Centers; 2016 [cited 2016 Sep 1]. Organophosphorus Insecticides: Dialkyl phosphate metabolites. Available from: https://www.cdc.gov/biomonitoring/OP-DPM_BiomonitoringSummary.html.
81. Margariti MG, Tsakalof AK, Tsatsakis AM. Analytical methods of biological monitoring for exposure to pesticides: Recent update. Ther Drug Monit. 2007;29(2):150–63, https://doi.org/10.1097/FTD.0b013e31803d3509.
82. Uzun FG, Kalender S, Durak D, Demir F, Kalender Y. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. Food Chem Toxicol. 2009;47(8):1903–8, https://doi.org/10.1016/j.fct.2009.05.001.

83. Narayana K, Prashanthi N, Nayanatara A, Bairy LK, D'Souza UJA. An organophosphate insecticide methyl parathion (o- o- dimethyl o-4-nitrophenyl phosphorothioate) induces cytotoxic damage and tubular atrophy in the testis despite elevated testosterone level in the rat. J Toxicol Sci. 2006;31(3):177–89, https://doi.org/10.2131/jts.31.177.
84. Narayana K, Prashanthi N, Nayanatara A, Kumar SG, Kumar HHC, Bairy KL, et al. A broad-spectrum organophosphate pesticide O,O-dimethyl O-4-nitrophenyl phosphorothioate (methyl parathion) adversely affects the structure and function of male accessory reproductive organs in the rat. Environ Toxicol Pharmacol. 2006;22(3):315–24, https://doi.org/10.1016/j.etap.2006.05.001.
85. Aguilar-Garduño C, Lacasaña M, Blanco-Muñoz J, Rodríguez-Barranco M, Hernández AF, Bassol S, et al. Changes in male hormone profile after occupational organophosphate exposure. A longitudinal study. Toxicology. 2013;307:55–65, https://doi.org/10.1016/j.tox.2012.11.001.
86. Khan IA, Reddy B V, Mahboob M, Rahman MF, Jamil K. Effects of phosphorothionate on the reproductive system of male rats. J Environ Sci Health B. 2001;36(4):445–56, https://doi.org/10.1081/PFC-100104188.
87. Penna-Videau S, Bustos-Obregón E, Cermeño-Vivas JR, Chirino D. Malathion affects spermatogenic proliferation in mouse. Int J Morphol. 2012;30(4):1399–407.
88. Recio-Vega R, Ocampo-Gómez G, Borja-Aburto VH, Moran-Martínez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: Association between sperm parameters and urinary pesticide levels. J Appl Toxicol. 2008;28(5):674–80, https://doi.org/10.1002/jat.1321.
89. Cocco P. On the rumors about the silent spring. Review of the scientific evidence linking occupational and environmental pesticide exposure to endocrine disruption health effects. Cad Saude Publica. 2002;18(2):379–402, https://doi.org/10.1590/S0102-311X2002000200003.
90. Bolognesi C. Genotoxicity of pesticides: A review of human biomonitoring studies. Mutat Res. 2003;543(3):251–72, https://doi.org/10.1016/S1383-5742(03)00015-2.
91. Mehrpour O, Karrari P, Zamani N, Tsatsakis AM, Abdollahi M. Occupational exposure to pesticides and consequences on male semen and fertility: A review. Toxicol Lett. 2014;230(2):146–56, https://doi.org/10.1016/j.toxlet.2014.01.029.
92. Senthilkumaran B. Pesticide- and sex steroid analogue-induced endocrine disruption differentially targets hypothalamo-hypophyseal-gonadal system during gametogenesis in teleosts – A review. Gen Comp Endocrinol. 2015;219:136–42, https://doi.org/10.1016/j.ygcen.2015.01.010.
93. Perry MJ, Venners SA, Chen X, Liu X, Tang G, Xing H, et al. Organophosphorous pesticide exposures and sperm quality. Reprod Toxicol. 2011;31(1):75–9, https://doi.org/10.1016/j.reprotox.2010.08.006.
94. Meeker JD, Stapleton HM. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ Health Perspect. 2010;118(3):318–23, https://doi.org/10.1289/ehp.0901332.
95. Melgarejo M, Mendiola J, Koch HM, Moñino-García M, Noguera-Velasco JA, Torres-Cantero AM. Associations between urinary organophosphate pesticide metabolite levels and reproductive parameters in men from an infertility clinic. Environ Res. 2015;137:292–8, https://doi.org/10.1016/j.envres.2015.01.004.
96. Figueroa ZI, Young HA, Meeker JD, Martenies SE, Barr DB, Gray G, et al. Dialkyl phosphate urinary metabolites and chromosomal abnormalities in human sperm. Environ Res. 2015;143:256–65, https://doi.org/10.1016/j.envres.2015.10.021.
97. Dobrzyńska MM. Phthalates – widespread occurrence and the effect on male gametes. Part 1. General characteristics, sources and human exposure. Rocz Panstw Zakl Hig. 2016;67(2):97–103.
98. Hauser R, Calafat AM. Phthalates and human health. Occup Environ Med. 2005;62(11):806–18, https://doi.org/10.1136/oem.2004.017590.
99. Annamalai J, Namasivayam V. Endocrine disrupting chemicals in the atmosphere: Their effects on humans and wildlife.

Environ Int. 2015;76:78–97, https://doi.org/10.1016/j.envint.2014.12.006.

100. Gray LE, Ostby J, Furr J, Price M, Veeramachaneni DNR, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci. 2000;58(2):350–65, https://doi.org/10.1093/toxsci/58.2.350.
101. Kay VR, Bloom MS, Foster WG. Reproductive and developmental effects of phthalate diesters in males. Crit Rev Toxicol. 2014;44(6):467–98, https://doi.org/10.3109/10408444.2013.875983.
102. Jurewicz J, Hanke W. Exposure to phthalates: Reproductive outcome and children health. A review of epidemiological studies. Int J Occup Med Environ Health. 2011;24(2):115–41, https://doi.org/10.2478/s13382-011-0022-2.
103. Wang Y-X, Zeng Q, Sun Y, Yang P, Wang P, Li J, et al. Semen phthalate metabolites, semen quality parameters and serum reproductive hormones: A cross-sectional study in China. Environ Pollut. 2016;211:173–82, https://doi.org/10.1016/j.envpol.2015.12.052.
104. Wang Y-X, You L, Zeng Q, Sun Y, Huang Y-H, Wang C, et al. Phthalate exposure and human semen quality: Results from an infertility clinic in China. Environ Res. 2015;142:1–9, https://doi.org/10.1016/j.envres.2015.06.010.
105. Wang Y-X, Zeng Q, Sun Y, You L, Wang P, Li M, et al. Phthalate exposure in association with serum hormone levels, sperm DNA damage and spermatozoa apoptosis: A cross-sectional study in China. Environ Res. 2015;150:557–65, https://doi.org/10.1016/j.envres.2015.11.023.
106. Liu L, Bao H, Liu F, Zhang J, Shen H. Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality, a primary study. Environ Int. 2012;42:78–83, https://doi.org/10.1016/j.envint.2011.04.005.
107. Kranvogl R, Knez J, Miuc A, Vončina E, Vončina DB, Vlaisavljević V. Simultaneous determination of phthalates, their metabolites, alkylphenols and bisphenol A using GC-MS in urine of men with fertility problems. Acta Chim Slov. 2014;61(1):110–20.
108. Pan Y, Jing J, Yeung LWY, Sheng N, Zhang H, Yao B, et al. Associations of urinary 5-methyl-2′-deoxycytidine and 5-hydroxymethyl-2′-deoxycytidine with phthalate exposure and semen quality in 562 Chinese adult men. Environ Int. 2016;94:583–90, https://doi.org/10.1016/j.envint.2016.06.020.
109. Pant N, Kumar G, Upadhyay AD, Patel DK, Gupta YK, Chaturvedi PK. Reproductive toxicity of lead, cadmium, and phthalate exposure in men. Environ Sci Pollut Res. 2014;21(18):11066–74, https://doi.org/10.1007/s11356-014-2986-5.
110. Pant N, Pant A, Shukla M, Mathur N, Gupta Y, Saxena D. Environmental and experimental exposure of phthalate esters: The toxicological consequence on human sperm. Hum Exp Toxicol. 2011;30(6):507–14, https://doi.org/10.1177/0960327110374205.
111. Jurewicz J, Radwan M, Sobala W, Ligocka D, Radwan P, Bochenek M, et al. Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones. Reprod Toxicol. 2013;42:232–41, https://doi.org/10.1016/j.reprotox.2013.10.001.
112. Axelsson J, Rylander L, Rignell-Hydbom A, Jönsson BAG, Lindh CH, Giwercman A. Phthalate exposure and reproductive parameters in young men from the general Swedish population. Environ Int. 2015;85:54–60, https://doi.org/10.1016/j.envint.2015.07.005.
113. Thurston SW, Mendiola J, Bellamy AR, Levine H, Wang C, Sparks A, et al. Phthalate exposure and semen quality in fertile US men. Andrology. 2016;4(4):632–8, https://doi.org/10.1111/andr.12124.
114. Bloom MS, Whitcomb BW, Chen Z, Ye A, Kannan K, Buck Louis GM. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum Reprod. 2015;30(11):2645–57, https://doi.org/10.1093/humrep/dev219.
115. Han X, Cui Z, Zhou N, Ma M, Li L, Li Y, et al. Urinary phthalate metabolites and male reproductive function

parameters in Chongqing general population, China. Int J Hyg Environ Health. 2014;217(2):271–8, https://doi.org/10.1016/j.ijheh.2013.06.006.

116. Specht IO, Toft G, Hougaard KS, Lindh CH, Lenters V, Jönsson BAG, et al. Associations between serum phthalates and biomarkers of reproductive function in 589 adult men. Environ Int. 2014;66:146–56, https://doi.org/10.1016/j.envint.2014.02.002.
117. Joensen UN, Frederiksen H, Blomberg Jensen M, Lauritsen MP, Olesen IA, Lassen TH, et al. Phthalate excretion pattern and testicular function: A study of 881 healthy Danish men. Environ Health Perspect. 2012;120(10):1397–403, https://doi.org/10.1289/ehp.1205113.
118. Meeker JD, Barr DB, Ryan L, Herrick RF, Bennett DH, Bravo R, et al. Temporal variability of urinary levels of nonpersistent insecticides in adult men. J Expo Anal Environ Epidemiol. 2005;15(3):271–81, https://doi.org/10.1038/sj.jea.7500402.
119. World Health Organization, Department of Reproductive Health and Research. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: The Organization; 2010.
120. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th ed. Cambridge: Cambridge University Press; 1999.